ESC European Society of Cardiology

Cardiovascular Research (2021) **117**, 1450–1488
doi:10.1093/cvr/cvaa324

REVIEW

Cardiac fibrosis

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Received 11 June 2020; editorial decision 6 October 2020; accepted 26 October 2020; online publish-ahead-of-print 2 November 2020

Abstract

Myocardial fibrosis, the expansion of the cardiac interstitium through deposition of extracellular matrix proteins, is a common pathophysiologic companion of many different myocardial conditions. Fibrosis may reflect activation of reparative or maladaptive processes. Activated fibroblasts and myofibroblasts are the central cellular effectors in cardiac fibrosis, serving as the main source of matrix proteins. Immune cells, vascular cells and cardiomyocytes may also acquire a fibrogenic phenotype under conditions of stress, activating fibroblast populations. Fibrogenic growth factors (such as transforming growth factor-β and platelet-derived growth factors), cytokines [including tumour necrosis factor-α, interleukin (IL)-1, IL-6, IL-10, and IL-4], and neurohumoral pathways trigger fibrogenic signalling cascades through binding to surface receptors, and activation of downstream signalling cascades. In addition, matricellular macromolecules are deposited in the remodelling myocardium and regulate matrix assembly, while modulating signal transduction cascades and protease or growth factor activity. Cardiac fibroblasts can also sense mechanical stress through mechanosensitive receptors, ion channels and integrins, activating intracellular fibrogenic cascades that contribute to fibrosis in response to pressure overload. Although subpopulations of fibroblast-like cells may exert important protective actions in both reparative and interstitial/perivascular fibrosis, ultimately fibrotic changes perturb systolic and diastolic function, and may play an important role in the pathogenesis of arrhythmias. This review article discusses the molecular mechanisms involved in the pathogenesis of cardiac fibrosis in various myocardial diseases, including myocardial infarction, heart failure with reduced or preserved ejection fraction, genetic cardiomyopathies, and diabetic heart disease. Development of fibrosis-targeting therapies for patients with myocardial diseases will require not only understanding of the functional pluralism of cardiac fibroblasts and dissection of the molecular basis for fibrotic remodelling, but also appreciation of the pathophysiologic heterogeneity of fibrosis-associated myocardial disease.

Keywords Fibrosis • Myofibroblast • Growth factor • Heart failure • Extracellular matrix

1. Introduction

The term fibrosis is used to describe the excessive deposition of extracellular matrix (ECM) proteins in parenchymal tissues, and typically reflects inappropriate or unrestrained activation of a reparative programme. Fibrosis is an important cause of organ dysfunction in many different diseases, including interstitial lung diseases, liver cirrhosis, progressive systemic sclerosis, and diabetic nephropathy.[1]

Myocardial fibrosis, the expansion of the cardiac interstitium due to net accumulation of ECM proteins, accompanies most cardiac pathologic conditions.[2,3] Although in most myocardial diseases, the extent of cardiac fibrosis predicts adverse outcome, fibrosis is not necessarily the primary cause of dysfunction. The adult mammalian heart has negligible regenerative capacity and heals through formation of a scar. Thus, in many cases, cardiac fibrosis is reparative, reflecting replacement of dead cardiomyocytes with a collagen-based scar. Myocardial infarction (MI) is a typical example of reparative fibrosis, as sudden death of a large number of cardiomyocytes stimulates inflammation and subsequent activation of reparative myofibroblasts, leading to formation of a scar. The scar lacks contractile capacity, but serves a critical protective role, maintaining the structural integrity of the chamber, and in the case of a transmural infarction, preventing catastrophic mechanical complications, such as cardiac rupture.[4] In other cardiac diseases, fibrosis predominantly involves the interstitium, and develops insidiously in the absence of significant cardiomyocyte loss. For example, systemic hypertension is associated with progressive interstitial and perivascular deposition of ECM proteins that increase myocardial stiffness and cause diastolic dysfunction. Ageing, obesity, and diabetes can also promote myocardial interstitial fibrosis

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and reduce ventricular compliance, potentially contributing to the pathogenesis of heart failure with preserved ejection fraction (HFpEF).

Thus, cardiac fibrosis is not a single disease entity that would predictably benefit from a standardized therapeutic intervention, but rather a pathologic response that may be appropriate or inappropriate, depending on the pathophysiologic context. This review article discusses the pathophysiologic heterogeneity of myocardial fibrotic responses, focusing on the functional consequences of cardiac fibrosis, the key cellular effectors, and the main molecular mechanisms. The article also highlights the unique characteristics of fibrotic remodelling in various myocardial diseases and discusses the benefits and perils of possible therapeutic interventions.

2. The normal cardiac interstitium

In adult mammalian hearts, cardiomyocytes occupy approximately 75% of the myocardial volume and are organized into laminae, 2–5 cells thick. These layers of cardiomyocytes are surrounded by an interstitial ECM network, comprised predominantly of fibrillar collagens.[5,6] The cardiac ECM not only serves as a mechanical scaffold but is also important for transmission of contractile force. In addition to type I collagen (the most abundant protein in the cardiac ECM) and type III collagen,[7,8] the cardiac ECM also contains a wide range of glycoproteins, glycosaminoglycans, and proteoglycans, and a reservoir of stored latent growth factors and proteases, that can be rapidly activated following injury to stimulate repair. Several different cell types are enmeshed within the interstitial ECM. Vascular endothelial cells are the most abundant non-cardiomyocytes in adult mouse hearts, reflecting the rich microvasculature of the myocardium.[9] Smaller populations of immune cells (macrophages, mast cells, and dendritic cells), and vascular mural cells (smooth muscle cells and pericytes) are also found in the normal cardiac interstitium.[10–13] Cardiac fibroblasts, the main matrix-producing cells, form one of the largest cell populations in normal mammalian hearts and are typically enmeshed in the endomysium and perimysium.[14,15] The abundance of fibroblasts in uninjured hearts may suggest an important homeostatic role; however, the functions of cardiac fibroblasts in the absence of injury remain poorly defined. Resident cardiac fibroblasts may serve to maintain the structural integrity of the matrix network,[16] regulating collagen turnover, a process that requires ongoing synthesis and degradation of ECM proteins.[17,18] Fibroblast-specific baseline activation of Smad3 signalling has been reported to maintain normal ECM content in adult mouse hearts,[19] whereas platelet-derived growth factor receptor (PDGFR)-α signalling was found to be important for fibroblast survival and optimal support of the normal microvascular network.[20] However, these actions had no significant effects on baseline organ function. It has been suggested that cardiac fibroblast subpopulations may also support cardiomyocyte survival and function; however, robust evidence documenting such actions in normal adult hearts is lacking. In uninjured mouse hearts, fibroblasts exhibit heterogeneity and can be subclassified into several subsets on the basis of their transcriptomic profile.[21] Whether these subsets exhibit distinct roles in cardiac homeostasis remains unknown.

3. Classification of myocardial fibrotic lesions

Basic histopathological analysis allows classification of cardiac fibrotic lesions into three distinct forms (*Figure 1*). In MI, necrotic cardiomyocytes are replaced by collagen-based scar, causing 'replacement fibrosis'. 'Interstitial fibrosis' describes the expansion of the endomysial and perimysial space, caused by net accumulation of ECM proteins in the absence of significant cardiomyocyte loss. The term 'perivascular fibrosis' is used to describe the expansion of the microvascular adventitia. This simple and crude classification has major implications regarding the pathophysiologic basis of the fibrotic lesion and its effects on cardiac function. Replacement fibrosis is typically reparative, reflecting the absence of regenerative capacity of the adult mammalian heart in response to primary necrotic injury. Interstitial and perivascular fibrosis on the other hand may result from prolonged activation of fibrogenic stimuli and may represent primary injurious processes. Perivascular fibrotic changes are prominent in hypertensive heart disease and may reflect fibroblast conversion or fibrogenic activation of vascular endothelial or mural cells,[22,23] or stimulation of adventitial fibroblasts.[24] Whether perivascular and interstitial fibrotic changes have distinct cell biological origins remains unknown. Replacement fibrosis is predominantly associated with systolic dysfunction. In contrast, interstitial and perivascular fibrosis typically reduce left ventricular compliance perturbing diastolic function with less pronounced or late effects on systolic function. Moreover, perivascular fibrosis may perturb coronary follow and induce microvascular dysfunction[25,26] precipitating diastolic dysfunction and worsening the severity of ischaemia.

More sophisticated analysis of the cellular composition, molecular and biochemical profile of fibrotic lesions could be used to gain additional information with mechanistic, diagnostic and potentially also prognostic significance. The relative abundance of immune cells, the phenotype and level of activation of fibroblasts and myofibroblasts, the expression of matricellular proteins and the levels of ECM cross-linking could provide key insights into the pathogenesis, activity and reversibility of myocardial fibrosis. In human patients with ischaemic cardiomyopathy undergoing aortocoronary bypass surgery, highly cellular interstitial fibrosis, characterized by increased inflammatory activity, and higher levels of the matricellular protein tenascin-C was associated with more dynamic lesions and with an increased likelihood of recovery after aortocoronary bypass surgery.[27,28] On the other hand, higher levels of ECM cross-linking and a hypocellular interstitium may mark fibrotic lesions with low potential reversibility.[29]

4. The cell biology of cardiac fibrosis

Activated fibroblasts are the central cellular effectors of myocardial fibrosis, serving as the main ECM-producing cells. Conversion of fibroblasts into secretory, matrix-producing and contractile cells, called myofibroblasts, is a crucial cellular event in many fibrotic conditions (*Figure 2*). Activated myofibroblasts are the main source of structural ECM proteins in fibrotic hearts,[31] produce large amounts of matricellular proteins,[32,33] and can also contribute to the regulation of matrix remodelling by producing proteases, such as matrix metalloproteinases (MMPs), and their inhibitors.[34] The expansion and activation of fibroblasts in remodelling hearts may involve direct effects of fibrogenic mediators, or upstream stimulation of other cell types, including immune cells, vascular cells and cardiomyocytes. Immune cells (macrophages, lymphocytes, mast cells, and eosinophils) may promote fibroblast activation by secreting cytokines, growth factors, and matricellular proteins. Vascular endothelial cells and pericytes may also secrete fibroblast-activating mediators, and have been reported to undergo myofibroblast conversion. Injured cardiomyocytes are also capable of producing

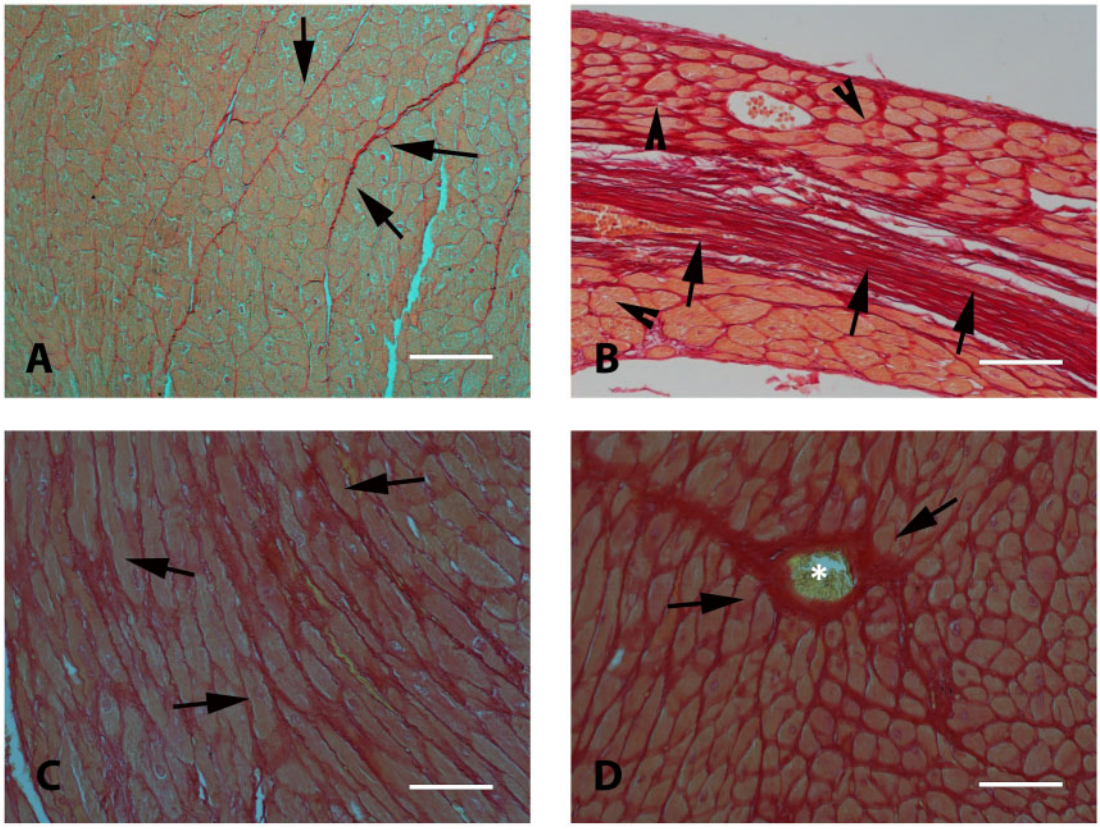

Figure 1 Histological types of cardiac fibrosis. Images show sections of adult mouse hearts stained with picrosirius red in order to label collagen fibres. (*A*) The normal adult mammalian heart contains an intricate network of collagenous matrix. Every cardiomyocyte is surrounded by a thin network of endomysial collagen. Thicker perimysial collagen fibres (arrows) enwrap bundles of cardiomyocytes. (*B*) Following myocardial infarction, dead cardiomyocytes are replaced by collagen-based scar. Replacement fibrosis is shown in a mouse model of reperfused myocardial infarction (arrows, 1 h ischaemia–7 days reperfusion). Infarction is midmyocardial; interstitial fibrosis is noted in remodelling subendocardial and subepicardial areas (arrowheads). (*C*) Interstitial fibrosis (arrows) is noted in a model of left ventricular pressure overload, induced through transverse aortic constriction (7 days). (*D*) Perivascular fibrosis (arrows) is also noted in the pressure-overloaded myocardium, with large amounts of collagen surrounding an intracoronary vessel (*). Scalebar = 80 μm.

fibrogenic mediators in response to stress, contributing to fibroblast activation. Moreover, in MI, death of cardiomyocytes and other myocardial cells results in release of damage-associated molecular patterns that activate inflammation, ultimately leading to fibrosis. The relative role of various cell types in the fibrotic response is dependent on the type of myocardial injury.

4.1 Fibroblasts and myofibroblasts: the central effector cells in cardiac fibrosis

The term fibroblast is used to describe cells of mesenchymal origin that populate connective tissues, lack a basement membrane and are capable of producing significant amounts of ECM proteins. Under this broad definition, a wide range of cardiac interstitial cells can be identified as fibroblasts. Fibroblasts can be distinguished from other myocardial interstitial cells on the basis of the expression of discoidin domain-containing receptor 2, PDGFR-α, and the transcription factor Tcf21.[15] Identification and characterization of fibroblast populations is further complicated by their dynamic phenotypic alterations in injured tissues.[35] In infarcted and stressed hearts, fibroblasts undergo conversion to myofibroblasts, cells that exhibit a prominent endoplasmic reticulum (indicating a synthetically active phenotype), while expressing contractile proteins, such as α-smooth muscle actin (α-SMA).[36,37] Infiltration of the heart with activated myofibroblasts has been reported in a wide range of pathologic conditions, including MI,[30,38] non-infarctive ischaemic cardiomyopathy,[27,39] myocarditis,[40] diseases associated with cardiac pressure or volume overload,[41,42] and alcoholic cardiomyopathy.[43] However, myofibroblast conversion is not required for activation of a pro-fibrotic programme. In a model of diabetic fibrotic cardiomyopathy, deposition of interstitial collagen and acquisition of a matrix-synthetic phenotype by cardiac fibroblasts was found to be independent of myofibroblast transdifferentiation.[44] Moreover, single-cell transcriptomic analysis in a model of angiotensin II-induced fibrosis identified fibroblast subsets expressing Cartilage Intermediate layer protein 1 (*Cilp*) or Thrombospondin-4 (*Thbs4*) as the predominant fibrogenic cell types, in the absence of α-SMA-expressing myofibroblasts.[45]

Moreover, a growing body of evidence suggests that myofibroblasts exhibit a range of phenotypic profiles. Although α-SMA expression is often used to identify 'differentiated myofibroblasts' in injured tissues, early stage myofibroblasts may not express α-SMA, but may have a robust

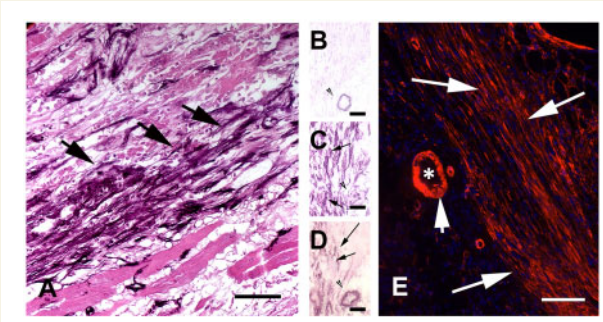

Figure 2 The myofibroblasts are the main cellular effectors of fibrosis in injured and remodelling hearts. Myofibroblasts are activated fibroblasts that express contractile proteins, such as α-SMA and secrete large amounts of ECM proteins. Images show abundant myofibroblasts in infarcted hearts in both large animal and rodent models. (*A*) Immunohistochemical staining identifies non-vascular α-SMA+ myofibroblasts in the border zone of a reperfused canine infarct (arrows, 1 h ischaemia/7 days reperfusion). (*B–D*) Serial staining of frozen sections from the infarcted canine heart show that border zone infarct myofibroblasts express α-SMA (*D*) and the embryonal isoform of smooth muscle myosin (SMemb) (*C*), but not the SM2 isoform, a marker of mature smooth muscle cells (*B*). Thus, these cells (arrows) are myofibroblasts and not smooth muscle cells. Arrowhead shows an arteriole, exhibiting expression of α-SMA (*D*) and SM2 (*B*). (*E*) Abundant α-SMA+ myofibroblasts are found in the border zone of a mouse infarct (7 days ischaemia). The short arrow points to the vascular smooth muscle cells on the arteriolar (*) media, which are also strongly positive for α-SMA. Scalebar = 80 μm. Original data published in ref.[30]

stress fibre network and form focal adhesions. These cells were termed 'proto-myofibroblasts',[46,47] and may be prominent in early interstitial fibrotic lesions triggered by mechanical stress. Transcriptomic analysis suggests that in myocardial injury sites, infiltration with activated fibroblasts expressing the matricellular protein periostin precedes their conversion to myofibroblasts.[21] Whether these activated periostin+/α-SMA- cells represent proto-myofibroblasts remains unknown.

In the era of single-cell transcriptomics, it is increasingly appreciated that both fibroblasts and myofibroblasts are heterogeneous populations, comprised of several subsets with distinct transcriptomic profiles. In healing infarcts subpopulations of myofibroblasts with high expression of fibrogenic mediators, such as transforming growth factor (TGF)-β, have been identified, along with other myofibroblasts that expressed transcripts encoding proteins with anti-fibrotic properties, such as the matricellular gene Wisp/CCN5 and the Wnt antagonist Sfrp.[21] Whether these transcriptomic profiles are associated with pro- vs. anti-fibrotic properties remains unknown.

4.2 Where do activated cardiac myofibroblasts come from?

The abundant fibroblasts that reside in the normal cardiac interstitium are capable of activation following injury and can account for the injury-associated expansion of matrix-secreting myofibroblasts. Despite this fact, several studies have suggested that other cell types, including haematopoietic fibroblast progenitors,[48–51] macrophages,[52] and endothelial cells[22,53] may also contribute to myofibroblast accumulation in injured hearts by transdifferentiating to fibroblasts or myofibroblasts. The relative contribution of these cells remains controversial. Several robust studies combining lineage tracing strategies, bone marrow transplantation experiments, and parabiosis models have demonstrated that the vast majority of activated myofibroblasts in both infarctive and in pressure overload-induced cardiac fibrosis are derived from resident fibroblast populations, and that the contributions of vascular endothelial cells and haematopoietic cells are very limited.[54–57] Several factors may explain the conflicting observations. First, some of the studies use associative data based on co-expression of various markers to imply haematopoietic or endothelial cell origin of fibroblasts. Second, in some studies the use of non-specific markers for identification of fibroblasts and myofibroblasts, such as fibroblast-specific protein-1 which is also highly expressed by macrophages and vascular cells,[58] may explain erroneous conclusions regarding cellular identity. Third, in some cases, the use of non-specific Cre drivers to trace endothelial cells or leucocytes may limit the value of the observations. Fourth, conflicting findings may reflect disease-specific and context-dependent alterations in the cellular composition of the cardiac interstitium. Generalizing cell biological paradigms to all types of myocardial injury are inherently problematic, and do not take into account the distinct profile of cellular responses caused by various injurious stimuli. For example, in inflammatory myocardial diseases, such as myocarditis, the intense chemokine-dependent infiltration of the heart with immune cells may also recruit abundant circulating progenitors that may contribute to fibroblast expansion, independently of the resident fibroblast population. Experiments using bone marrow chimeric mice in a model of autoimmune myocarditis suggested that a large fraction of activated fibroblasts originate from a population of prominin+ haematopoietic cells that infiltrate the myocardium.[59] On the other hand, in MI, contributions of various cell types may be context-dependent. In a reperfused infarct, most fibroblasts in the area at risk likely survive the ischaemic event and may account for myofibroblast expansion. In contrast, when the coronary artery is permanently occluded, the majority of cardiac interstitial cells may die along with the neighbouring cardiomyocytes; thus, limiting their contribution to the reparative myofibroblast population, which may be derived predominantly from fibroblasts recruited from non-infarcted segments, or from other remote cell types (including circulating haematopoietic cells). Unfortunately, studies on myocardial cell death following infarction have focused almost exclusively on cardiomyocytes; information on the fate of fibroblasts and other interstitial cells is extremely limited. The unique cell biological characteristics of disease-specific fibrotic myocardial responses remain underappreciated and understudied.

4.3 Mechanisms involved in myofibroblast activation in fibrotic hearts

Activated myofibroblasts contribute to matrix remodelling, not only by secreting large amounts of structural ECM proteins but also by releasing proteases and their inhibitors and by producing enzymes involved in matrix processing. Although the mechanisms of myofibroblast activation are dependent on the specific type of injury causing the myocardial fibrotic response, some common patterns have emerged. Neurohumoral mediators, cytokines and growth factors are released and activated following myocardial injury and bind to cell surface receptors, transducing fibrogenic intracellular signalling cascades. Moreover, specialized matrix proteins and matricellular macromolecules enrich the matrix network and serve to locally activate fibrogenic growth factors at the site of injury providing spatial regulation of the fibroblast-activating signals.

In addition to the fibroblasts, several other cell types contribute to the fibrogenic environment. Immune cells, including macrophages, mast cells, and lymphocytes are recruited and activated in remodelling hearts and may play an important role in fibroblast activation by secreting a wide range of fibrogenic mediators, including cytokines, growth factors, and matricellular proteins.[60–65] Endothelial cells, pericytes, and vascular smooth muscle cells may also secrete molecular signals that regulate fibroblast behaviour.[66] Cardiomyocytes are also highly sensitive to microenvironmental changes and typically respond to injurious stimuli by activating inflammatory and fibrogenic programmes.[67] It should be emphasized that the actions of leucocytes, vascular cells and cardiomyocytes on fibroblast phenotype are not necessarily activating. Several studies have demonstrated injury-associated induction and release of anti-fibrotic mediators in leucocytes,[68] endothelial cells,[69,70] and cardiomyocytes[71] that may serve to restrain fibrogenic responses.

5. Fibroblast-activating neurohumoral pathways

5.1 The renin–angiotensin–aldosterone system

5.1.1 The angiotensin II/AT1 axis

The renin–angiotensin–aldosterone system is activated in fibrotic and remodelling hearts regardless of the underlying aetiology and plays an important role in myofibroblast activation. Stressed cardiomyocytes, immune cells and fibroblasts produce renin and angiotensin-converting enzyme (ACE), contributing to angiotensin II generation[72–74] (*Figure 3*). When released in the cardiac interstitium, angiotensin II is a potent activator of cardiac fibroblasts. *In vitro* studies have demonstrated that angiotensin II stimulates cardiac fibroblast proliferation,[75] inhibits fibroblast apoptosis,[76] stimulates fibroblast migration[77,78] inducing integrin expression,[79] promotes myofibroblast conversion,[80] and markedly increases synthesis of structural ECM proteins.[81,82] All these activating effects of angiotensin II involve signalling through the type 1 angiotensin receptor (AT1). In contrast, the type 2 receptor AT2 acts as a negative regulator of angiotensin II-mediated fibrogenic responses, inhibiting AT1 actions and suppressing fibroblast proliferation and matrix synthesis.[83,84]

The activating effects of the angiotensin II/AT1 axis in cardiac fibroblasts are mediated at least in part through activation of p38 mitogen-activated protein kinase (MAPK), protein kinase C (PKC) and extracellular signal-regulated kinase (Erk) cascades.[75,85–87] Some fibrogenic angiotensin II actions require induction,[88,89] or activation of TGF-β.[90] Moreover, responsiveness of fibroblasts to angiotensin II is modulated by a wide range of mediators that regulate expression of the angiotensin receptors. For example, pro-inflammatory cytokines may make fibroblasts more responsive to the effects of angiotensin II by inducing AT1 synthesis.[91] In contrast, angiotensin II has been reported to downmodulate AT1 expression,[92] providing a negative feedback mechanism that may restrain uncontrolled fibrogenic actions.

There is abundant *in vivo* evidence, predominantly derived from pharmacologic interventions, supporting the role of AT1 signalling in the pathogenesis of cardiac fibrosis. AT1 inhibition attenuated interstitial fibrosis in models of MI[93] and left ventricular pressure overload.[94] Despite the lack of evidence documenting the role of fibroblast-specific AT1 signalling *in vivo* using cell-specific genetic approaches, the potent *in vitro* effects of the angiotensin II/AT1 axis suggest that cardiac fibroblasts may be major targets of angiotensin II. Thus, the beneficial effects of ACE inhibition and AT1 blockade in heart failure patients may reflect, at least in part, attenuation of angiotensin-mediated fibrosis. Evidence in isolated cardiomyocytes has suggested that AT1 signalling may not always require angiotensin II stimulation but may be directly activated by mechanical stress, stimulating a hypertrophic response.[95] Whether angiotensin-independent AT1 signalling is involved in myofibroblast activation and in the pathogenesis of cardiac fibrosis remains unknown.

5.1.2 The fibrogenic actions of aldosterone

Extensive evidence suggests that aldosterone may play an important role in fibroblast activation following myocardial injury.[96,97] In isolated cardiac fibroblasts, aldosterone triggers a proliferative response,[98] stimulates migration,[99] and promotes a matrix-synthetic phenotype,[100] at least in part through activation of MAPK cascades.[101] Whether these actions are responsible for the fibrogenic actions of aldosterone *in vivo* is unclear. In addition to its effects on fibroblasts, aldosterone has been suggested to modulate cardiomyocyte, macrophage, lymphocyte, and vascular cell phenotype, leading to activation of a fibrogenic programme. Experiments using cell-specific mineralocorticoid receptor knockout mice suggested that aldosterone actions on myeloid cells,[102] T lymphocytes,[103] and cardiomyocytes[104] may contribute to the pathogenesis of cardiac fibrosis.

5.2 The β-adrenergic response in fibroblast activation

Adrenergic stimulation activates fibroblasts and induces fibrotic cardiac remodelling. *In vivo*, infusion of the non-specific β adrenergic receptor (AR) agonist isoproterenol,[105] or the α1 AR agonist phenylephrine,[106] and transgenic overexpression of β2-ARs[107] cause marked myocardial fibrosis. The pro-fibrotic effects of adrenergic stimulation may be indirect, reflecting reparative fibrosis in response to cardiomyocyte necrosis, release of fibrogenic mediators by stimulated cardiomyocytes, or immune cell activation.[105,108–110] Although direct effects of adrenergic agonists on cardiac fibroblasts are well-documented, their *in vivo* significance is unclear. β-AR activation stimulates proliferation of cardiac fibroblasts,[111,112] activating phosphatidylinositol 3-kinase (PI-3K)[113] and MAPK[114] signalling Moreover, proliferative effects of β-AR stimulation in fibroblasts have been attributed to epidermal growth factor receptor (EGFR) transactivation and downstream activation of ERK signalling.[115]

The relative role of β1- and β2-AR responses in regulating the fibrotic response is unknown. Although in some experimental animal models of heart failure, non-selective β-AR inhibition and administration of β1-AR blockers attenuated cardiac fibrosis,[116,117] such effects may represent an epiphenomenon reflecting reduced blood pressure or attenuated cardiomyocyte death. Moreover, β2-AR signalling in myofibroblasts has been reported to exert pro-hypertrophic actions through paracrine mechanisms.[118] Although the role of β3-AR signalling in fibroblasts is unknown, recent evidence suggests that cardiomyocyte-specific β3-AR signalling may exert anti-fibrotic actions by downmodulating expression of cardiomyocyte-derived fibrogenic proteins, such as the matricellular protein CCN2.[119]

AR signalling induces conformational changes in G protein βγ subunits, ultimately resulting in activation of G protein-coupled receptor kinase 2 (GRK2). Activation of GRK2 in fibroblasts has been implicated in cardiac fibrosis in experimental models of MI.[120,121] However, the molecular targets responsible for the fibrogenic actions of GRK2 remain poorly understood.

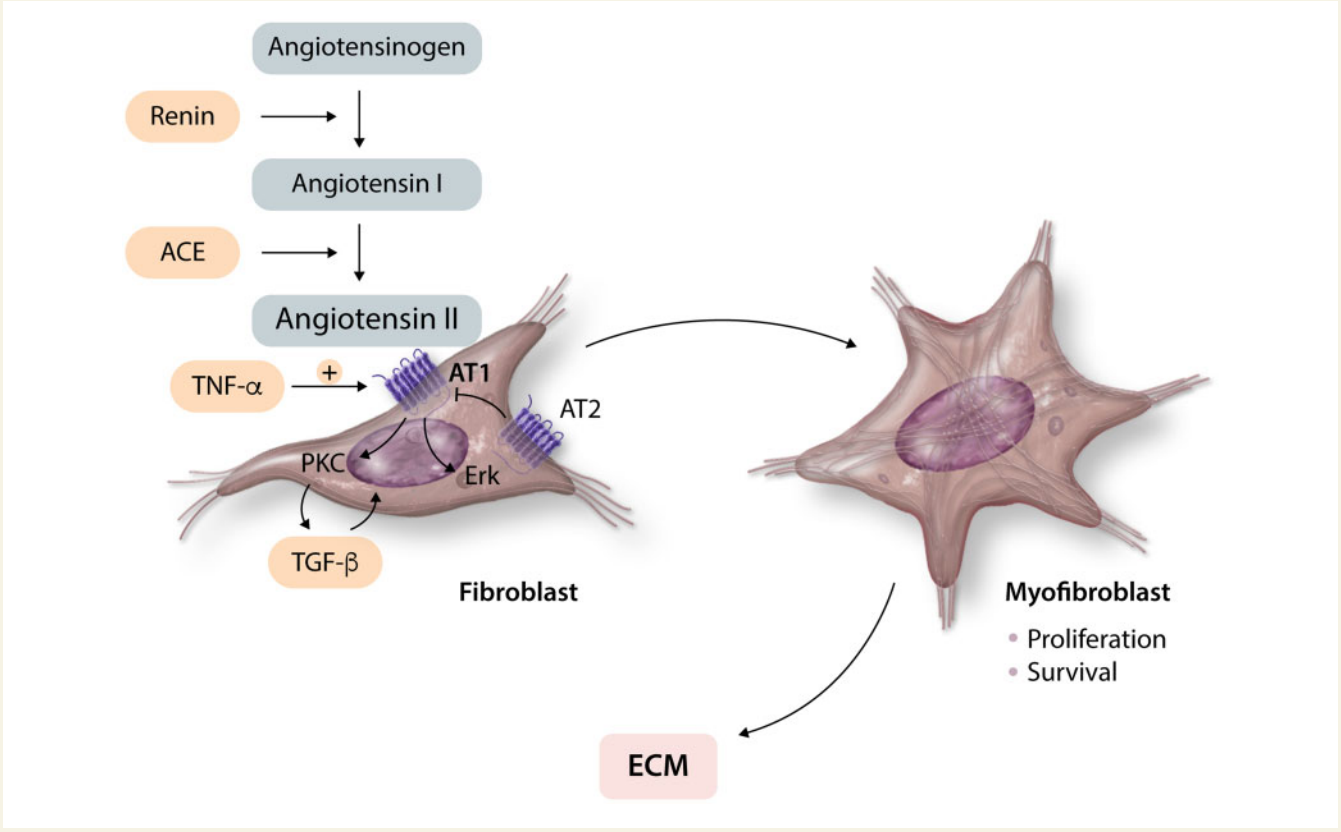

Figure 3 Fibrogenic effects of angiotensin II. Angiotensin II generated in injured or remodelling myocardium exerts potent fibrogenic actions on cardiac fibroblasts through interactions with the AT1 receptor, promoting myofibroblast conversion, fibroblast proliferation and survival and stimulating ECM synthesis. In contrast, signalling through the AT2 receptor inhibits the pro-fibrotic effects of AT1. Pro-inflammatory cytokines, such as TNF-α, induce AT1 in cardiac fibroblasts and may accentuate the activating effects of angiotensin II. Fibrogenic effects of angiotensin II are mediated through activation of PKC and Erk signalling. Some of the effects of angiotensin II may involve downstream induction and activation of TGF-β.

5.3 Inflammatory mediators as regulators of fibroblast function

Inflammatory cytokines, chemokines, and growth factors have been implicated in the pathogenesis of cardiac fibrosis through direct actions on fibroblasts, by stimulating recruitment and activation of fibrogenic macrophages and lymphocytes, and by triggering a fibrogenic programme in vascular cells and cardiomyocytes. Moreover, prolonged chronic inflammation may cause cardiomyocyte necrosis, triggering a reparative form of fibrosis.

5.3.1 Chemokines as regulators of cardiac fibrosis

Chemokines are chemotactic cytokines with a prominent role in leucocyte trafficking.[122] From a structural perspective, chemokines are classified into four subfamilies (CC, CXC, CX3C, and XC), on the basis of the number of aminoacids between their first two cysteine residues. This classification has important functional implications: CC chemokines are predominantly mononuclear cell chemoattractants, whereas CXC chemokines, and in particular the subgroup containing the conserved ELR sequence motif (glutamic acid–leucine–arginine) near the aminoternimal end, are involved in recruitment of neutrophils. Actions of chemokines in regulation of fibrosis are predominantly mediated through chemotactic recruitment of leucocytes. Although several members of the family may directly modulate fibroblast phenotype and function, the *in vivo* significance of these effects in cardiac fibrotic remodelling remains unclear.[123]

5.3.2 CC chemokines

Extensive experimental evidence suggests that the CC chemokine CCL2/monocyte chemoattractant protein-1 is involved in the pathogenesis of cardiac fibrosis in a wide range of models, including ischaemic cardiomyopathy, pressure overload-induced myocardiac remodelling and inflammatory heart diseases (*Table 1*).[125,126,129] The fibrogenic actions of CCL2 has been predominantly attributed to recruitment and activation of monocytes and macrophages expressing the main CCL2 receptor, CCR2, resulting in up-regulation of fibrogenic mediators, such as TGF-β and osteopontin.[128,129,137] Although CCL2 has been suggested to promote direct fibrogenic actions in hepatic, cutaneous and pulmonary fibroblasts,[138–140] in isolated cardiac fibroblasts, no significant effects of CCL2 were noted on profibrotic gene expression profile and proliferative activity.[129] Some studies have suggested that CCL2 may promote fibrosis by recruiting circulating fibroblast progenitors.[49,50] However, the evidence supporting this notion is associative and based on co-expression of haematopoietic cell and fibroblast markers by the same cells. Considering the robust evidence from several studies using lineage tracing strategies suggesting that most activated fibroblasts in the remodelling heart are derived from resident populations and not from

Table 1 The CCL2/CCR2 axis in cardiac fibrosis

Experimental model of cardiac fibrosis	Findings	Cellular target and molecular mechanism	References
Transgenic CCL2 overexpression	Cardiomyocyte-specific CCL2 overexpression in mice induced myocardial inflammation and fibrotic changes.	Fibrogenic actions presumed due to inflammatory cell recruitment and activation.	124
Rat model of cardiovascular remodelling through chronic inhibition of NO synthesis	Treatment with anti-CCL2 antibody attenuated inflammation and reduced coronary vascular remodelling but did NOT affect fibrosis.	NA	125
Rat model of pressure overload through suprarenal aortic constriction	Anti-CCL2 antibody attenuated myocardial fibrosis.	Anti-fibrotic effects were presumed due to decreased macrophage recruitment and attenuated TGF-β release	126
Mouse model of non-reperfused myocardial infarction	Anti-CCL2 gene therapy attenuated interstitial fibrosis without affecting infarct size.	Anti-fibrotic effects were attributed to decreased macrophage infiltration and attenuated TNF-α and TGF-β levels.	127
Mouse model of reperfused myocardial infarction	CCL2 KO mice had attenuated myofibroblast proliferation and delayed granulation tissue formation after MI, associated with reduced dilative remodelling.	Anti-fibrotic effects were attributed to reduced macrophage recruitment and impaired macrophage activation, associated with markedly decreased expression of TGF-β and osteopontin.	128
Mouse model of ischaemic cardiomyopathy induced through brief repetitive ischaemia/reperfusion	CCL2 KO mice had attenuated interstitial fibrosis, associated with reduced macrophage recruitment and decreased fibroblast proliferation.	CCL2 did not have direct effects on proliferation or expression of fibrosis-associated genes by cardiac fibroblasts. Thus, the profibrotic effects of CCL2 were attributed to actions on macrophages.	129
Mouse model of angiotensin II-induced fibrosis	CCL2 KO mice had attenuated fibrosis.	Fibrogenic effects of CCL2 were attributed to recruitment of circulating fibroblast precursors. This notion was based on the presence of CD34+/CD45+ collagen-expressing fibroblast-like cells in the myocardium.	50
Mouse model of angiotensin II-induced fibrosis	CCR2 KO mice had attenuated angiotensin II-mediated cardiac fibrosis.	Fibrogenic effects of CCR2 were attributed to recruitment of bone marrow-derived fibroblast precursors. This notion was based on the presence of CD34+/CD45+ collagen-expressing fibroblast-like cells in the myocardium.	130
Mouse model of angiotensin II-induced fibrosis	CCR2 KO mice had attenuated angiotensin II-mediated cardiac fibrosis.	Fibrogenic effects of CCR2 were attributed to attenuated recruitment of fibroblast progenitors. This notion was based on detection of CD45/α-SMA+ cells. CCR2-mediated fibrosis was attributed to actions of CCL12 (and not CCL2), based on associative data.	131
Mouse model of mineralocorticoid receptor-mediated cardiac fibrosis	In a deoxycorticosterone (DOC)/salt model, CCL2 KO mice had 35% less myocardial fibrosis.	Fibrogenic effects of CCL2 were attributed to actions on macrophage recruitment and activation. However, CCL2 KO mice also had lower blood pressure, suggesting that the fibrogenic actions of CCL2 may be indirect, reflecting effects on blood pressure regulation.	132
Mouse model of ageing	Aged CCL2 KO mice had reduced fibrosis and attenuated diastolic dysfunction.	Attenuated fibrosis in CCL2 KO mice was associated with reduced leucocyte infiltration.	133
Mouse model of angiotensin-induced fibrosis	Administration of a CCR2 antagonist attenuated myocardial inflammation and fibrosis.	CCL2 up-regulation was stimulated by angiotensin-induced CaMKIIδ activation. The mechanisms for the fibrogenic effects of the CCL2/CCR2 axis were not investigated.	134
Mouse model of left ventricular pressure overload	Depletion of CCR2+ monocytes ameliorated cardiac fibrosis.	The findings highlighted the role of the monocytes recruited through CC chemokines in the pathogenesis of myocardial fibrosis.	135
Mouse model of streptozotocin-induced diabetes	Diabetic CCR2 KO mice had attenuated myocardial fibrosis.	The fibrogenic actions were attributed to macrophage-driven inflammation and oxidative stress.	136

haematopoietic cells[55–57] the role of circulating fibroblast progenitors in cardiac fibrosis remains controversial.[141]

5.3.3 CXC chemokines

Several members of the CXC chemokine family have been implicated in the pathogenesis of cardiac fibrosis. CXC chemokines that bind to the CXCR2 receptor may exert fibrogenic actions through recruitment of neutrophils or monocytes with fibrogenic properties. In spontaneously hypertensive rats, CXCR2 inhibition was found to attenuate fibrosis[142]; however, CXCR2-mediated fibrosis may involve effects on blood pressure regulation, rather than pro-fibrotic actions. CXCL1, one of the CXCR2 ligands has been reported to contribute to the development of angiotensin-induced cardiac fibrosis through recruitment of a fibrogenic monocyte subpopulation.[143]

CXCL12 has a broad range of actions in injured and remodelling hearts and has been involved in angiogenesis, recruitment of inflammatory and progenitor cells, and in regulation of cardiomyocyte survival following injury.[144,145] Several studies have suggested that CXCL12 may exert fibrogenic actions through CXCR4 activation. CXCR4 antagonism reduced cardiac fibrosis in a genetic model of murine cardiomyopathy,[146] and in models of diabetic fibrosis[147] and cardiorenal syndrome.[148] The fibrogenic actions of CXCL12 were attributed to direct pro-migratory effects on fibroblasts, to recruitment of fibroblast progenitors, or to activation of macrophages.[149]

The ELR-negative CXC chemokines do not stimulate neutrophil chemotaxis, but recruit lymphocyte subsets and have been suggested to exert actions on fibroblasts and endothelial cells. The ELR-CXC chemokine CXCL10/interferon-γ-inducible protein (IP)-10 is consistently up-regulated following cardiac injury and has been suggested to exert anti-fibrotic actions. Inhibition of fibrosis by CXCL10 may involve recruitment of yet unidentified leucocyte subsets that suppress fibroblast function, or direct de-activating effects on cardiac fibroblasts.[69,70] *In vitro*, CXCL10 was found to inhibit growth-factor-mediated fibroblast migration,[69] through interactions with proteoglycans that were independent of the main CXCL10 receptor, CXCR3.[150]

5.3.4 The pro-inflammatory cytokines in cardiac fibrosis

Levels of the pro-inflammatory cytokines tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6 are markedly increased in many myocardial pathologic conditions associated with fibrosis. Several clinical studies have demonstrated correlations between pro-inflammatory cytokine levels and fibrosis-related endpoints. For example, in patients with dilated cardiomyopathy, elevated myocardial IL-6 and TNF-α mRNA levels were associated with collagen deposition and gelatinase expression,[151] and in transplanted human hearts myocardial IL-6 levels were associated with fibrotic remodelling.[152] These associations suggest a link between pro-inflammatory cytokines and fibroblast activation. The fibrogenic effects of TNF-α, IL-1, and IL-6 may involve direct actions on fibroblasts, or indirect effects, related to recruitment of macrophages, induction of matricellular proteins and up-regulation of growth factors with prominent fibroblast-activating properties, such as TGF-β.

5.3.5 TNF-α

Abundant *in vivo* evidence suggests an important role for TNF-α in cardiac fibrosis (*Table 2*). Transgenic mice with myocardial TNF-α overexpression develop heart failure associated with fibrotic changes.[153,154] The time course of ECM remodelling in TNF-α overexpressing hearts suggests that early protease-mediated matrix degradation is followed by late up-regulation of TGF-βs and activation of a matrix-preserving programme.[155] Experiments using loss-of-function models have implicated endogenous TNF-α in the pathogenesis of fibrosis induced through pressure overload[157,163] through actions involving the type 1 TNF receptor (TNFR1).[161]

TNF-α may promote fibrosis through direct actions on cardiac fibroblasts, or through effects on other myocardial cell types. It should be noted that TNF-α does not directly induce a matrix-synthetic programme in cardiac fibroblasts, but rather decreases collagen synthesis and stimulates MMP expression.[164] Thus, TNF-α-mediated fibrosis may reflect a response to ECM degradation. *In vitro* experiments have suggested several additional mechanisms that may be responsible for TNF-α-induced fibroblast activation. TNF-α stimulated synthesis of the matricellular protein CCN4 and subsequent fibroblast proliferation,[165] induced TGF-β expression,[166] and may potentiate fibrogenic effects of angiotensin II, by stimulating AT1 receptor synthesis.[91] TNF-α may also promote fibrogenic activation of immune cell populations in the injured myocardium. In TNF-α- overexpressing mice, the fibrogenic actions of TNF-α in the myocardium have been attributed, at least in part, to expansion of cardiac mast cells.[160]

5.3.6 The fibrogenic actions of the IL-1 family of cytokines

Studies in experimental models of MI have implicated IL-1 in the pathogenesis of cardiac fibrosis. Global loss of IL-1 signalling in mice lacking the type 1 IL-1 receptor (IL-1R1) attenuated adverse fibrotic remodelling in a model of reperfused MI.[167] Moreover, in a model of viral myocarditis local inhibition of IL-1 through overexpression of IL-1 receptor antagonist (IL-1Ra) significantly reduced fibrosis.[168] Much like for TNF-α, the fibrogenic actions of IL-1 likely involve effects in both fibroblasts and immune cells. Experiments in animals with fibroblast-specific loss of the IL1R1 receptor documented the significance of direct actions of IL-1 on cardiac fibroblasts. Fibroblast-specific IL-1R1 KO mice had lower myocardial collagen levels in the infarcted heart, and exhibited improved function and attenuated remodelling following MI.[169] *In vitro* studies suggest that the fibrogenic effects of IL-1 are not mediated through direct activation of myofibroblast-driven ECM synthesis. In fact, both IL-1α and IL-1β inhibit conversion of fibroblasts into myofibroblasts,[170–172] inhibit fibroblast proliferation,[173] and decrease collagen synthesis by cardiac fibroblasts.[164] IL-1-mediated fibrosis may be due to primary activation of pro-inflammatory and matrix-degrading pathways. IL-1 potently stimulates MMP expression and activity,[167,170] while reducing synthesis of MMP inhibitors.[174] Generation of matrix fragments and induction of TGF-βs by IL-1 may be responsible for indirect activation of a fibrogenic programme.

IL-33, another member of the IL-1 family of cytokines has been implicated in the regulation of fibrosis in several different organs.[175] In human patients with end-stage heart failure, IL-33 and ST2 up-regulation were associated with fibrotic changes.[176] In the pressure-overloaded myocardium, IL-33 was found to exert cardioprotective and anti-fibrotic actions, attributed to its binding to the ST2 receptor in cardiomyocytes and fibroblasts.[177] However, considering the potent effects of the IL-33/ST2 axis on cardiomyocytes, macrophages and lymphocytes, whether the protective effects of IL-33 in the remodelling heart are mediated through direct actions on fibroblasts remains unknown. *In vitro*, IL-33 stimulation did not affect collagen synthesis by cardiac fibroblasts, but inhibited their migratory capacity and enhanced expression of inflammatory genes.[178]

Table 2 The role of TNF-α in cardiac fibrosis

Model	Findings	Cellular mechanism of fibrosis	References
Cardiac-specific TNF-α overexpression	Terminally ill mice with myocardial overexpression of TNF-α had biventricular fibrosis. This was accompanied by chamber dilation, cardiomyocyte apoptosis, and transmural myocarditis.	Not studied. TNF-induced fibrosis may reflect activation of immune cells that release fibrogenic cytokines, cardiomyocyte death activating a reparative programme, or direct effects of TNF-α on fibroblasts.	[153]
Cardiac-specific TNF-α overexpression	Cardiac overexpression of TNF-α caused heart failure associated with cardiac fibrosis.	Fibrosis was associated with marked induction of collagen synthesis and activationg of matrix-degrading proteases (MMP2 and MMP9). The primary cellular targets of TNF-α are unclear.	[154]
Cardiac-specific TNF-α overexpression	Cardiomyocyte-specific TNF-α overexpression caused progressive ventricular dilation and fibrosis.	Early activation of matrix-degrading MMPs was followed by late increases in TGF-β levels and fibrillar collagen content. Thus, TNF-α may promote an early matrix-degrading response, followed by a late TGF-β-driven matrix-preserving process.	[155]
Global TNFR1 and TNFR2 KO mice undergoing non-reperfused MI protocols.	Interstitial fibrosis was increased in TNFR2, but not in TNFR1 KO mice. Infarct size was comparable between groups.	Unclear cellular targets. TNFR2 loss was associated with increased post-infarction IL-1β and IL-6 levels that may account for fibrogenic actions.	[156]
TNF-α KO mice undergoing transverse aortic constriction (TAC) protocols	TNF-α loss attenuated cardiac fibrosis following TAC, associated with improved cardiac function.	TNF-α mediates fibrosis and protease activation in the pressure-overloaded heart. Cellular targets unclear.	[157]
Anti-TNF monoclonal antibody treatment in streptozotocin-induced diabetic cardiomyopathy	TNF-α inhibition reduced myocardial fibrosis and improved LV function.	TNF-α mediates fibrosis activating a matrix-synthetic programme. Cellular targets unclear.	[158]
TNF-α KO mice undergoing TAC protocols	TNF loss attenuated cardiac fibrosis following TAC.	Profibrotic effects of TNF-α were attributed to activation of a superoxide/MMP axis more prominently in cardiomyocytes and at a lower level in fibroblasts.	[159]
Cardiac-specific TNF-α overexpression	TNF overexpression was associated with mast cell expansion and subsequent fibroblast activation. Mast cell deficiency abrogated TNF-induced fibrosis.	Profibrotic effects of TNF-α may involve mast cell growth and activation, and subsequent release of mast-cell-derived fibrogenic mediators.	[160]
TNFR1 and TNFR2 KO mice undergoing angiotensin II infusion protocols	TNFR1 loss, but not TNFR2 absence, attenuated cardiac fibrosis.	Fibrogenic actions of TNF-α/TNFR1 signalling were attributed to recruitment of circulating fibroblast progenitors. This notion was based on identification of CD34+/CD45+ fibroblast-like cells.	[161]
Mdx dystrophic mice receiving anti-TNF treatment (Remicade)	Remicade attenuated cardiac fibrosis but worsened systolic function.	The basis for the effects of TNF blockade was not studied.	[162]
TNF-α KO mice undergoing angiotensin II infusion protocols	TNF-α KO mice had reduced perivascular and interstitial fibrosis following angiotensin II infusion.	Fibrogenic actions were attributed to effects on oxidative stress-dependent MAPK, TGF-β, and nuclear factor (NF)-κB signalling. Fibrogenic effects may be in part related to hypertensive actions of TNF-α.	[163]

5.3.7 The IL-6 family of cytokines

The IL-6 family of cytokines (also referred as the gp130 family, indicating the common signal transducer of these molecules) includes IL-6, IL-11, oncostatin-M, leukaemia-inhibitory factor and cardiotrophin-1 and has been implicated in the pathogenesis of cardiac fibrosis. The bulk of experimental evidence suggests that IL-6 exerts fibrogenic actions. Genetic absence of IL-6 attenuated cardiac fibrosis and dysfunction in models of left ventricular pressure overload,[179,180] MI,[181] and diabetic cardiomyopathy.[182] IL-6 neutralization has also been reported to exert anti-fibrotic actions. In models of aldosterone-mediated cardiac remodelling, left ventricular pressure overload, and genetic cardiomyopathy induced through Hsp20 overexpression, IL-6 neutralization reduced fibrotic changes and attenuated fibroblast activation.[183–185] The fibrogenic effects of IL-6 have been attributed to STAT3-dependent stimulation of collagen synthesis by cardiac fibroblasts,[186] or to induction of TGF-β.[182]

Recent studies have suggested that IL-11 may play a critical role in the pathogenesis of fibrosis not only in the myocardium[187] but also in the lung and kidney,[188,189] serving as a downstream fibrogenic signal, induced by TGF-β. IL-11 inhibition attenuated TGF-β-mediated fibroblast activation *in vitro*, and global loss of IL-11Ra *in vivo* reduced interstitial fibrotic

remodelling following pressure overload. The effects of IL-11 on fibroblast activation were attributed to post-transcriptional mechanisms involving ERK signalling. To what extent IL-11 exerts pro-fibrotic actions in human patients remains unclear. Recombinant human IL-11 has been extensively used for the treatment of thrombocytopenias with a good safety record and without any reports of fibrotic disease.[190]

It should be emphasized that studies examining the role of other gp130 cytokines do not support a consistent fibrogenic role of gp130 signalling. Oncostatin M has been suggested to exert anti-fibrotic actions in the myocardium by inhibiting fibrogenic TGF-β signalling cascades.[191] Experimental studies in fibrotic responses of other organs have produced conflicting findings suggesting both pro-fibrotic[192] and anti-fibrotic effects[193] of oncostatin M. The conflicting data likely reflect the context-dependent actions of gp130 cytokines *in vivo*, which are driven by the wide range of their actions in many different cell types.

5.3.8 The fibrogenic actions of the anti-inflammatory cytokines IL-4, IL-10, and IL-13

The anti-inflammatory cytokines IL-4, IL-10, and IL-13 have been implicated in the pathogenesis of fibrosis in several different organs.[194,195] Extensive *in vivo* and *in vitro* evidence supports the role of endogenous IL-4 in cardiac fibrosis. IL-4 up-regulation has been reported in experimental models of cardiac injury, predominantly localized in mast cells,[196] lymphocytes, and macrophages. In myocarditis, eosinophils may be an additional major source of IL-4.[197] In a model of angiotensin II infusion, genetic loss of IL-4 reduced collagen deposition in the myocardium by 60%.[198] Moreover, in a model of pressure overload induced through TAC, IL-4 neutralization attenuated fibrotic changes.[199] IL-4 may exert direct fibrogenic actions by stimulating collagen synthesis in cardiac fibroblasts through activation of STAT6.[198]

In contrast, the evidence on the role of IL-13 in cardiac fibrosis is more limited. Induction of IL-13 has been documented in infarcted[200] and in fibrotic senescent mouse hearts[201] and may be localized in Th2 lymphocytes. IL-13 can activate a matrix-synthetic programme in fibroblasts,[202] stimulating TGF-β expression and activation,[203] and may promote a fibrogenic phenotype in monocytes and macrophages.[201] However, the *in vivo* contribution of these fibrogenic actions of IL-13 in the cardiac fibrotic response has not been documented.

IL-10 up-regulation has been documented in both rodent and large animal models of reparative and pressure overload-induced fibrosis and is localized in T lymphocytes and subsets of macrophages infiltrating the injured and remodelling heart.[204–206] In addition to its well-documented anti-inflammatory actions, IL-10 may also regulate the cardiac fibrotic response. Unfortunately, data from *in vivo* studies are conflicting, suggesting both pro-fibrotic and anti-fibrotic actions of IL-10. In a model of cardiac remodelling induced through unilateral nephrectomy, salt loading and aldosterone infusion, IL-10 was found to critically contribute to fibroblast activation and to the pathogenesis of diastolic dysfunction.[206] The fibrogenic effects of IL-10 were indirect, and were attributed to acquisition of a fibrogenic programme by macrophages. These findings are consistent with *in vitro* observations suggesting that IL-10 does not significantly affect fibroblast gene expression profile,[207] but promotes a matrix-preserving phenotype in macrophages through up-regulation of TIMP-1 synthesis.[204] In contrast, other studies suggested that endogenous IL-10 may not significantly contribute to fibrosis, or may even exert anti-fibrotic actions. In a model of reperfused infarction, genetic loss of IL-10 had no significant effects on scar formation, myofibroblast infiltration and adverse remodelling.[207] In a model of non-reperfused MI, IL-10 was found to inhibit fibrosis.[208] Moreover, in a model of pressure overload, endogenous IL-10 derived from haematopoietic cells inhibited fibrosis, through actions attributed to attenuated maturation of fibroblast progenitors.[209] What is the basis for the conflicting evidence? The *in vivo* effects of IL-10 in the myocardial fibrotic response may be dependent on the balance between anti-inflammatory and pro-fibrotic actions. IL-10-mediated suppression of pro-inflammatory cytokine expression may inhibit downstream activation of fibrogenic pathways and, depending on the context, may outweigh any pro-fibrotic actions.

5.4 The TGF-β superfamily

5.4.1 TGF-*β*s in cardiac fibrosis

TGF-β is the best characterized fibrogenic growth factor.[210,211] In mammals, the TGF-β subfamily is comprised of three isoforms (TGF-β1, 2, and 3)[212] that signal through the same receptors and share common cellular targets, but exhibit distinct patterns of regulation and different affinities for their receptors and co-receptors. Thus, the three isoforms likely play different roles in the pathophysiology of fibrotic conditions. Unfortunately, isoform-specific actions have not been systematically studied *in vivo*. Thus, our knowledge on the role of TGF-β in fibrotic conditions is primarily derived from studies investigating the common downstream signalling pathways of the three isoforms. TGF-β induction and activation has been consistently demonstrated in several different models of cardiac fibrosis,[205,213,214] and in human hearts with fibrotic cardiomyopathic changes.[215,216]

Although many different cell types (including macrophages, fibroblasts, cardiomyocytes, and platelets) can produce *de novo* large amounts of TGF-β in the injured heart, TGF-β signalling in injured tissues is predominantly regulated through activation. The mammalian heart contains latent stores of TGF-β that cannot associate with TGF-β receptors. Injury results in rapid activation of TGF-β, providing a mechanism for appropriate spatially-restricted stimulation of the reparative TGF-β response. Pericellular activation of TGF-β in sites of injury involves several different molecular signals, including proteases,[217–219] matricellular proteins,[214,220] and integrins.[221] The relative contribution of specific TGF-β activating signals in the fibrotic heart has not been investigated.

The role of TGF-β in the pathogenesis of cardiac fibrosis is supported by several lines of evidence. First, gain-of-function studies showed that myocardial overexpression of TGF-β1, and constitutive expression of an activated mutant TGF-β protein trigger ventricular fibrosis, associated with *de novo* deposition of structural ECM proteins and activation of a matrix-preserving programme.[222–224] Surprisingly, transgenic mice with a large proportion of constitutively active TGF-β1 due to a mutation that blocks covalent tethering of the TGF-β1 latent complex to the ECM exhibited only atrial fibrosis,[225] possibly reflecting exaggerated responses of atrial fibroblasts to the fibrogenic effects of TGF-β. Second, genetic loss-of-function studies suggest that endogenous TGF-β signalling plays an important role in cardiac fibrosis. Heterozygous TGF-β1+/- deficient mice had delayed interstitial fibrotic changes as they aged.[226] Moreover, mice with fibroblast-specific loss of the type I or type II TGF-β receptors (TβRI or TβRII) had reduced cardiac fibrosis in a model of left ventricular pressure overload[227] suggesting that fibrotic remodelling involves the activation of the TGF-β signalling cascade. Third, pharmacologic TGF-β inhibition strategies attenuated fibrosis in a model of pressure overload,[228] and in the fibrotic cardiomyopathy due to Chagas' disease.[229]

TGF-β exerts a broad range of direct effects on fibroblasts that may contribute to the pathogenesis of fibrosis. TGF-β stimulation potently induces myofibroblast conversion,[230] and increases ECM

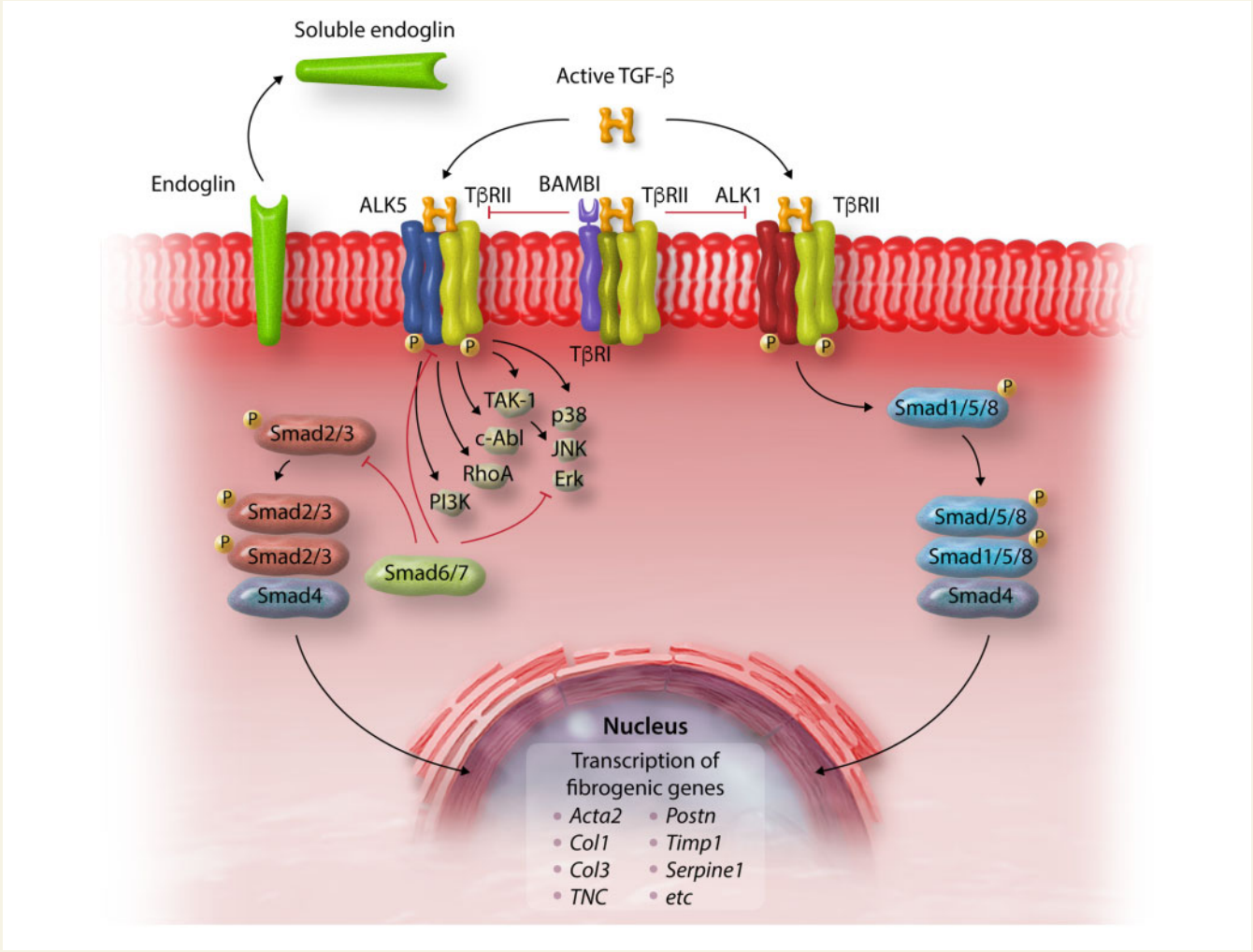

Figure 4 Regulation of TGF-β signalling in cardiac fibrosis. Active TGF-β binds to type II and type I receptors, activating downstream Smad-dependent signalling cascades and Smad-independent pathways. TGF-β binding to the ALK5 type 1 receptor and downstream activation of Smad3 signalling, induces a matrix-preserving programme in cardiac fibroblasts and plays an important role in their activation following cardiac injury. In contrast, the role of ALK1/Smad1/5 signalling in regulation of fibroblast phenotype is poorly understood. Activation of Smad-independent pathways, including RhoA and MAPK signalling, mediates some of the effects of TGF-β in cardiac fibroblasts. Endogenous pathways for negative regulation of TGF-β cascades may protect from excessive or unrestrained fibrotic responses. The inhibitory Smads (Smad6/7), pseudoreceptors such as BAMBI, and soluble endoglin may serve as endogenous inhibitors of TGF-β signalling, limiting pro-fibrotic responses.

protein synthesis by activated fibroblasts.[231] Moreover, TGF-β increases expression of integrins,[232] and shifts the protease/anti-protease balance towards a matrix-preserving phenotype by inducing protease inhibitors, such as Plasminogen Activator Inhibitor (PAI)-1 and tissue inhibitor of metalloproteinase (TIMP)1[212,233] and by suppressing MMP synthesis.[234] It should be noted that the effects of TGF-β on cardiac fibroblast proliferation are dependent on the context. Some studies have reported that TGF-β stimulates proliferation of cardiac fibroblasts,[235] whereas other investigations demonstrated anti-proliferative effects.[231,236] The contrasting findings likely reflect differences in the state of differentiation of fibroblast populations,[237] the presence or absence of other growth factors, and the matrix environment.

Considering the broad effects of TGF-β in many cellular responses, and the need to develop strategies to specifically inhibit its fibrogenic actions, dissection of pro-fibrotic TGF-β-driven molecular signals has attracted significant interest. TGF-β signals through a series of intracellular effector proteins, the Smads, and through Smad-independent pathways (*Figure 4*). *In vitro* and *in vivo* evidence suggests that both canonical Smad-dependent cascades and non-canonical Smad-independent signalling contribute to fibroblast activation in fibrotic hearts. *In vivo* studies have demonstrated that Smad3 signalling is critically involved in activation of reparative fibroblasts following MI, inducing ECM protein synthesis, integrin transcription, and α-SMA expression,[231,233] and contributing to the formation of an organized scar.[232] Smad3 signalling in infarct myofibroblasts restrained cell proliferation,[231–233] generating well-aligned arrays of activated myofibroblasts that preserve the structural integrity of the infarcted heart through activation of a reparative integrin-reactive oxygen species (ROS) axis.[232] These findings highlight the reparative function of fibroblasts in the presence of significant cardiomyocyte loss. In contrast, Smad2 does not seem to play a major role in mediating fibroblast activation in fibrotic hearts.[238] The contrasting effects of Smad2 and

Smad3 may reflect differences in their patterns of activation and nuclear translocation following TGF-β stimulation, or distinct interactions with transcriptional regulators in the nucleus. On the other hand, some actions of TGF-β on cardiac fibroblasts may involve activation of Smad-independent p38 MAPK, ERK, or Transforming Growth Factor-β-activated kinase (TAK)1 signalling.[114,239,240] However, considering the wide range of mediators that signal through these common kinase pathways, the relative contribution of TGF-β in their activation is unclear.

In addition to the direct actions of TGF-β on transcription of fibrosis-associated genes, some TGF-β-mediated fibrogenic effects may be mediated through induction of other secreted mediators. It has been suggested that TGF-β-mediated fibrosis may require downstream release of the matricellular protein CCN2/connective tissue growth factor.[241] However, experiments using both loss- and gain-of-function approaches have challenged the *in vivo* significance of CCN2 in mediating the pro-fibrotic actions of TGF-β.[224,242] The gp130 cytokine IL-11 has also been suggested to act as a critical downstream signal mediating TGF-β-driven fibrosis not only in the heart[187] but also in other organs.[188,189]

Considering its broad cellular targets, some of the *in vivo* fibrogenic actions of TGF-β may involve indirect activation of other cell types. Direct *in vivo* evidence supporting this notion is lacking. Although myeloid cell-specific loss of Smad3 markedly attenuated TGF-β synthesis by macrophages, collagen deposition in the infarct and interstitial fibrosis in non-infarcted areas was not affected in a model of non-reperfused MI.[243]

5.4.2 Other members of the TGF-*β* superfamily

Information on the role of other members of the TGF-β superfamily in cardiac fibrosis is limited. Several members of the bone morphogenetic protein (BMP) subfamily, including BMP2, BMP4, and BMP6, are up-

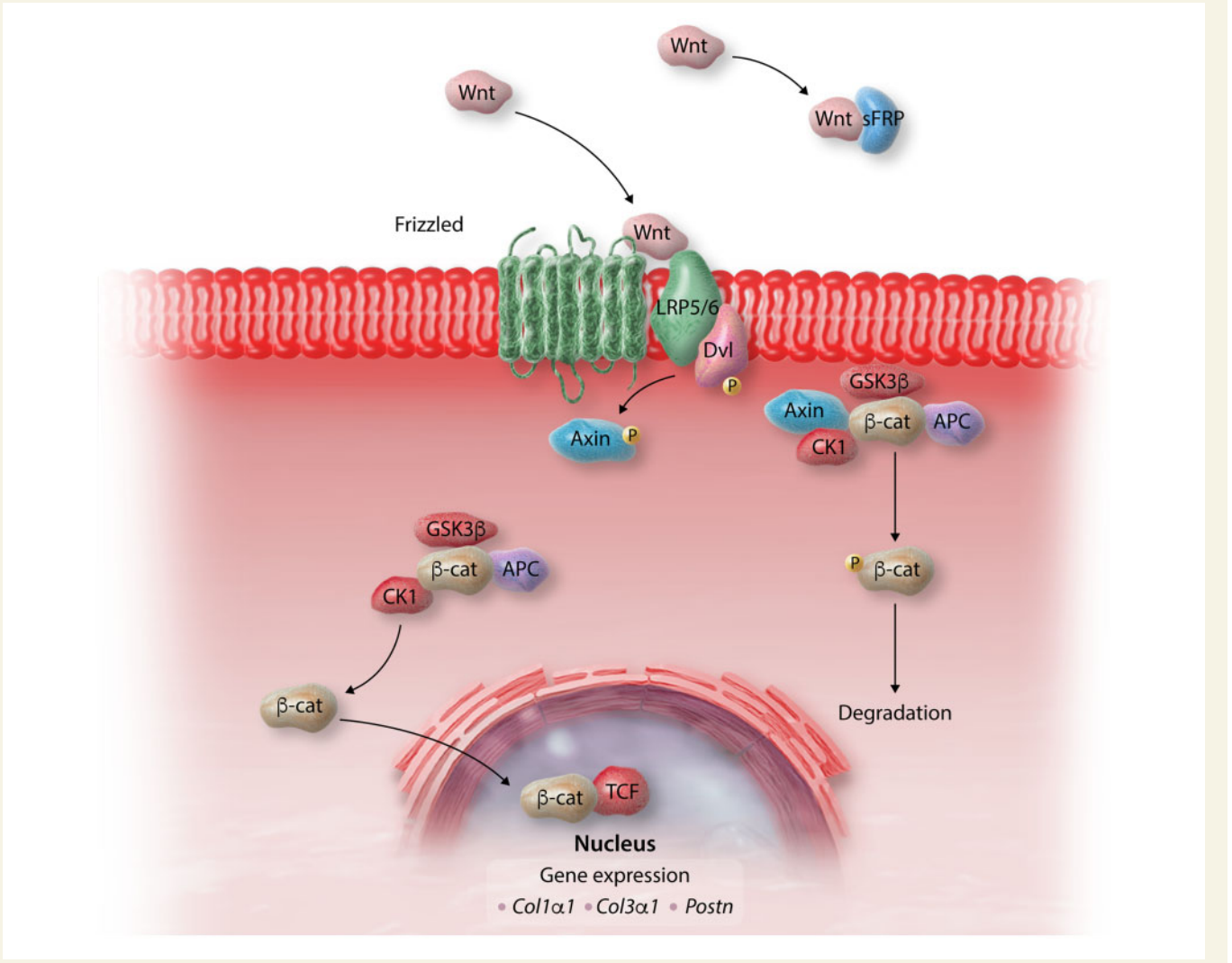

Figure 5 The Wnt/β-catenin pathway plays an important role in cardiac fibrosis. In the absence of Wnt, cytoplasmic β-catenin is continuously degraded by the Axin complex, which involves casein kinase 1 (CK1), adenomatous polyposis coli (APC) and glycogen synthase kinase 3 (GSK3). Wnts are released in injured and remodelling myocardium and bind to Frizzled transmembrane receptors and their co-receptors LRP5/6. This complex recruits the scaffolding protein Dishevelled (Dvl) and inhibits axin-mediated β-catenin phosphorylation, which is required for ubiquitination and proteasomal degradation. Thus, through the effects of Wnts, β-catenin is stabilized and translocates to the nucleus, where it interacts with T cell Factor (TCF), regulating gene expression. Activation of β-catenin in cardiac fibroblasts has been implicated in fibrogenic signalling and in paracrine hypertrophic effects on cardiomyocytes. Secreted frizzled-related proteins (sFRP) bind to Wnts and inhibit their actions.

regulated in infarcted hearts and may exert pro-inflammatory actions[244]; however, their potential involvement in the fibrotic response has not been investigated. Two members of the BMP subfamily, BMP7 and BMP10, have been reported to exert anti-fibrotic effects. Several independent investigations suggested that BMP7 administration attenuates collagen deposition in models of pressure overload and diabetic cardiomyopathy.[22,245,246] The antifibrotic effects were attributed to blockade of TGF-β signalling and were associated with attenuated endothelial to mesenchymal transition.[22,247] Moreover, gain-of-function studies have suggested that BMP10 may exert anti-fibrotic actions in the isoproterenol-treated heart.[248] Whether these effects involve direct modulation of fibroblast phenotype, or indirect actions involving other cell types is unclear.

The follistatins bind with high affinity with TGF-β superfamily proteins (such as activins, BMPs and growth differentiation factors) disrupting their function. In addition to these effects, several members of the follistatin family have been implicated in the pathogenesis of cardiac fibrosis through direct activation of cardiac fibroblasts. Follistatin-like 1 activated a reparative programme in infarct fibroblasts through ERK signalling and protected the infarcted ventricle from cardiac rupture.[249] *In vitro* studies have suggested that follistatin-like 3 may also have fibroblast-activating effects.[250]

5.5 The PDGFs

The PDGFs (PDGF-AA, -BB, AB, CC, and DD) are homo- or heterodimeric growth factors that signal through two different receptors: PDGFR-α and PDGFR-β. Abundant evidence suggests that PDGF isoforms play a role in activation of the fibrotic response following myocardial injury. PDGF-A, PDGF-C, and PDGF-D have potent fibrogenic effects on the myocardium, mediated through direct actions and, at least in part, through up-regulation of TGF-β.[251] *In vitro*, PDGF-A and PDGF-D potently stimulate cardiac fibroblast proliferation and ECM protein synthesis.[252,253] *In vivo*, overexpression of PDGF-A or PDGF-D cause marked fibrotic cardiomyopathy associated with fibroblast activation.[254,255] PDGF isoforms and PDGFRs are up-regulated in fibrotic hearts[256] and have been implicated in the pathogenesis of fibrotic responses. Consistent with its high expression in fibroblasts, PDGFR-α activation has been consistently involved in myocardial fibrosis. In a model of reperfused MI, treatment with a neutralizing anti-PDGFR-α antibody attenuated collagen deposition.[256] Moreover, in pressure-overloaded hearts, PDGFR-α blockade reduced atrial fibrosis and protected from atrial fibrillation.[257] Although such effects likely reflect direct inhibition of PDGFR - α-driven fibroblast activation, actions on other cell types cannot be excluded. Moreover, broad PDGFR blockade through administration of the non-specific small molecule kinase inhibitor imatinib was found to reduce interstitial fibrosis in models of MI[258] and viral myocarditis.[259]

The PDGF-BB/PDGFR-β axis on the other hand is predominantly activated in vascular mural cells; thus, its fibrogenic actions may be more limited. PDGFR-β activation through an integrin β1-mediated mechanism was suggested to augment fibroblast proliferation and matrix synthesis in a model of cardiac pressure overload.[260] However, mice overexpressing PDGF-B had only focal fibrotic changes, much less severe than PDGF-A overexpressing animals.[255] Neutralization of PDGFR-β signalilng in infarcted mice reduced collagen deposition, but had much more impressive effects in perturbation of vascular maturation, highlighting the significance of PDGFRβ actions on vascular mural cells.[256,261]

5.6 The Wnt/β-catenin axis

In mammals, the Wnt signalling pathway is involved in embryonic development, but becomes quiescent in adult low turnover tissues, such as the heart. Members of the Wnt family of secreted glycoproteins are secreted following injury,[53,262] bind to Frizzled family transmembrane receptors and initiate signalling through canonical pathways involving β-catenin, or through non-canonical pathways. Activation of the canonical β-catenin pathway requires Wnt-mediated dissociation of the β-catenin degradation complex, stabilization and accumulation of β-catenin in the cytoplasm, followed by nuclear translocation and interactions with transcription factors and transcriptional co-activators that regulate gene expression (*Figure 5*). Wnt/β-catenin activation has broad effects on a wide range of cellular responses,[263] involved in repair, remodelling and regeneration.

In the injured heart, Wnt signalling is activated in many different cell types and has been suggested to contribute to reparative, regenerative and fibrotic responses. Secretion of Wnt proteins may be mediated through growth factors, such as TGF-β,[40] and requires post-translational modification by the acyltransferase Porcupine (Porcn), a process that supplies a single fatty acid adduct necessary for Wnt protein secretion. *In vitro*, Wnt family members promote conversion of cardiac fibroblasts to myofibroblasts and stimulate cytokine synthesis.[264,265] *In vivo*, administration of a small molecule inhibitor that disables Porcn, thus preventing secretion of Wnt proteins, reduced fibrosis in a model of MI.[266] Wnt-mediated fibroblast activation in the injured heart likely involves signalling through the β-catenin pathway.[267,268] Cell-specific genetic loss-of-function studies demonstrated that fibroblast-specific activation of β-catenin mediates both fibrosis and cardiomyocyte hypertrophy in the pressure-overloaded heart.[268] The downstream molecular targets of the Wnt/β-catenin axis remain unknown. It should be emphasized that Wnt-induced fibrogenic actions may not be due exclusively to direct fibroblast activation, but may also involve effects on other cell types, including cardiomyocytes, vascular cells, and immune cells.[269,270]

5.7 Endothelin-1

Endothelin (ET)-1 exerts potent fibrogenic effects, acting downstream of cytokines and neurohumoral mediators,[271] thus linking inflammation and fibrosis.[272] Angiotensin II and TGF-β induce ET-1 *in vitro*,[273] and in experimental models of cardiac fibrosis.[274] Up-regulation of ET-1 is consistently noted in many fibrosis-associated cardiac pathologies, including MI, heart failure, hypertensive heart disease, diabetes, and ageing.[275–277] Fibrogenic effects of ET-1 in myocardial disease are suggested by both genetic models and by pharmacologic inhibition studies. Cardiac ET-1 overexpression in mice induced myocardial fibrosis associated with both systolic and diastolic dysfunction.[278] Moreover, endothelial-specific loss of ET-1 attenuated fibrosis in a model of angiotensin II infusion.[279] In experimental animal models of hypertensive heart disease and of reparative myocardial fibrosis, ET-1 inhibition reduced fibrotic remodelling.[280,281] The anti-fibrotic effects of ET-1 blockade may have therapeutic implications. Endothelin receptor antagonists reduced myocardial stiffness, suppressing collagen I deposition and attenuating interstitial and perivascular fibrosis in rat and mouse models of aldosterone infusion that recapitulate features of human HFpEF.[282,283]

To what extent the fibrogenic effects of ET-1 are due to direct activation of fibroblasts vs. indirect actions on other cellular targets, such as endothelial cells and cardiomyocytes, remains unknown. *In vitro*, cardiac fibroblasts are highly responsive to ET-1 stimulation, exhibiting increased proliferative activity,[284] accentuated ECM protein synthesis,[285] and

Table 3 Functions of the prototypical matricellular proteins in cardiac fibrosis

Matricellularprotein	Biochemical properties	Proposed functions in models of cardiac remodelling and fibrosis	References
Thrombospondin (TSP)-1	Homotrimeric glycoprotein with multiple functional domains. Characterized by the presence of three properdin-like repeats, called type 1 domains.	TGF-β activation and subsequent myofibroblast conversion. Inhibition of MMPs and stabilization of the matrix network. Inhibition of angiogenesis. Direct effects on collagen fibril formation.	65,214,326–328
Tenascin C	Hexameric multimodular glycoportein (hexabrachion). Not expressed in normal hearts, but induced and deposited in remodelling myocardium.	Pro-inflammatory actions. Recruitment of fibrogenic macrophages. Activation of fibrogenic integrin signalling inmacrophages. Fibrogenic actions though accentuation of growth factor signalling. Regenerative actions in fish and amphibians.	329–334
SPARC (secreted protein acidic and rich in cysteine)	Multimeric glycoprotein with a high-capacity calcium-binding domain, a follistatin-like domain and high affinity to collagen.	Regulates post-synthetic collagen processing. May exert fibrogenic actions through ADAMTS1 induction. Accentuates growth factor signalling, promoting fibroblast activation and angiogenesis.	335–338
Osteopontin	Acidic glycoprotein expressed predominantly in macrophages, but also in other cell types, with a central RGD sequence that binds integrins. Also interacts with CD44 through RGD-independent mechanisms.	Regulates matrix assembly. Activates growth factor signalling, promoting fibroblast activation and myofibroblast conversion. May stimulate integrin-dependent fibrogenic actions. May induce MMPs. May induce expression and activity of the cross-linking enzyme lysyl-oxidase. May induce galectin-3 expression in macrophages. Has been suggested to exert fibrogenic actions through effects on mitochondrial function.	339–348
Periostin	Fasciclin domain-containing adhesive glycoprotein, highly expressed by activated fibroblasts and secreted into the ECM following injury.	Regulates matrix assembly. Activates a fibrogenic programme in fibroblasts. May have regenerative functions in neonatal mouse hearts.	349–351

resistance to apoptosis.[286] In a model of type 1 diabetes, ET-1-mediated EndMT has been suggested to contribute to the myocardial fibrotic response.[287] Unfortunately, the evidence supporting this specific cellular mechanism was limited to dual staining with non-specific markers for fibroblasts and endothelial cells.

5.8 The ECM as a modulator of the fibrosis-associated cellular response

The profound changes in the amount and biochemical profile of the ECM proteins in fibrotic hearts, not only has consequences on cardiac function and conduction of the electrical impulse, but may also play a major role in regulation of cellular responses and in transduction of fibrogenic signalling pathways. Generation of matrix fragments (termed matricryptins and matrikines) by activated proteases ,[288] deposition of provisional matrix proteins (such as fibronectin and fibrin), and induction of matricellular macromolecules that enrich the interstitial matrix are the best-documented mechanisms for matrix-mediated regulation of fibrogenic cellular responses in injured hearts. Cardiac remodelling is intricately regulated by this crosstalk between the cells and the surrounding ECM: the cells secrete ECM molecules with regulatory properties, but also respond to changes in the matrix environment by interacting with specific functional domains of ECM macromolecules.[65,289]

5.8.1 Matrikines and matricryptins

Many forms of cardiac injury, including myocardial ischaemia and pressure overload, induce rapid release and activation of proteases that degrade the native ECM, generating bioactive peptides termed matrikines and matricryptins.[290,291] Matrikines are fragments of ECM macromolecules with biological properties distinct from those of the full-length form of the molecule. The term matricryptin is used to describe a matrikine that requires proteolytic processing to expose a bioactive functional domain.[292] These matrix fragments bind to cell surface receptors on fibroblasts, vascular cells or immune cells, and can modulate the fibrotic response. In a protease-rich environment, all constituents of the cardiac ECM, (including collagens I, IV, and XVIII, elastin, fibronectin, hyaluronan, and tenascin-C) can generate fragments.[288,293] Most of the evidence on the role of ECM fragments in tissue injury suggests actions that regulate

angiogenesis, and effects on immune cells that activate inflammatory signalling cascades,[291,294] and may indirectly promote fibrosis. In addition to these actions, *in vitro* studies have demonstrated that matrix fragments may also modulate fibroblast phenotype, potentially exerting direct actions in cardiac fibrosis.[295–298] However, the *in vivo* role of specific matrix fragments in fibrotic myocardial conditions has not been investigated.

5.8.2 The provisional matrix components: fibrin and fibronectin

Myocardial injury increases microvascular permeability, causing extravasation of plasma fibrinogen and fibronectin and generating a provisional matrix network that is particularly prominent during the inflammatory and proliferative phase of MI.[299] The provisional matrix provides a dynamic substrate for interactions between integrins expressed by fibroblasts and immune cells, and functional domains of fibrin and fibronectin, thus facilitating cell migration and transducing activating signals.[300,301] The ED-A variant of cellular fibronectin plays an important role in activation of TGF-β and in subsequent myofibroblast conversion.[47,302–304] Moreover, the plasma-derived provisional matrix is later enriched with cell-derived matrix components, including non-fibrillar collagens, cellular fibronectin, proteoglycans and a wide range of matricellular macromolecules.

5.8.3 Deposition and cross-linking of fibrillar collagens

Secretion of the major fibrillar collagens, collagen I and collagen III is the hallmark of cardiac fibrosis.[8,31] The relative amounts of type I vs type III collagen fibres may be important in regulation of the mechanical properties of the myocardium. Collagen I fibres are thicker and stiffer; in contrast the finer reticular collagen III fibres are more compliant. The accentuated and prolonged up-regulation of type I collagen in hypertensive hearts has been suggested to increase myocardial stiffness.[305,306] Moreover, in patients with dilated cardiomyopathy enrichment of the cardiac ECM with collagen I fibres was associated with reduced ventricular compliance.[307] Other members of the fibrillar collagen subfamily may play important regulatory roles in cardiac fibrosis. Collagen V is known to regulate collagen fibril diameter and assembly. Recent studies in a model of MI suggested that collagen V induction in healing scars restrains fibroblast activation by regulating expression of mechanosensitive integrins.[308]

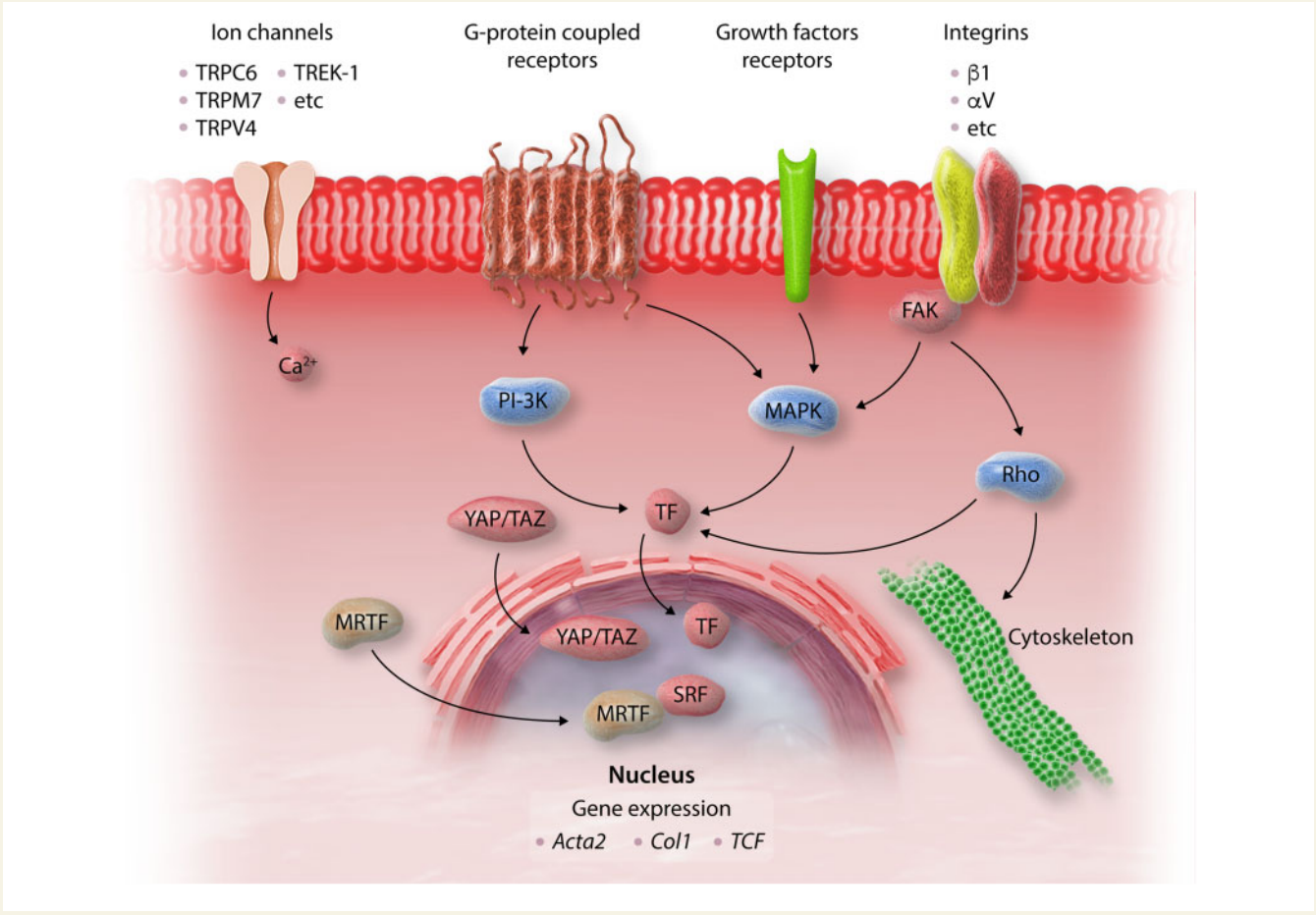

Figure 6 Mechanosensitive pathways play a critical role in fibroblast activation in many cardiac pathologic conditions. Integrins, mechanosenstive ion channels, growth factor receptor activation, and activation of G-protein coupled receptors stimulate FAK, MAPK, RhoA/ROCK, and PI-3K signalling, mediating responses of fibroblasts to mechanical stress. Cytoskeletal changes are also sensed through the MRTF/SRF axis or the YAP/TAZ system and regulate transcription of fibrogenic genes. TF, transcription factors.

Following their secretion by activated fibroblasts and myofibroblasts, procollagen chains are processed, assembled into fibrils and cross-linked. Cross-linking enzymes, such as the members of the lysyl-oxidase (LOX) and transglutaminase (TG) families play a crucial role in formation of intermolecular covalent links between collagen chains. The LOX family is the main enzymatic system responsible for cross-linking of extracellular collagen in fibrotic hearts[309–313]; its members act on specific lysine or hydroxylysine residues, catalysing allysine aldehyde formation, leading to subsequent spontaneous formation of the cross-links. TG2, (also known as tissue transglutaminase) is the highest-expressed member of the transglutaminase family in remodelling hearts[314–316] and catalyzes formation of an isopeptide bond between the ε-amino group of a lysine and the γ-carboxamide group of a glutamine. In addition to its enzymatic effects, TG2 may also regulate fibroblast phenotype through non-enzymatic mechanisms, modulating integrin-dependent responses.[317] Cross-linking is a major determinant of collagen fibre stiffness and resistance to degradation and has major implications in regulation of cardiac function.[318] Although some matrix cross-linking activity is required to provide mechanical support and to prevent chamber dilation under conditions of stress[319] excessive collagen cross-linking increases myocardial stiffness and can contribute to the pathogenesis of diastolic dysfunction.[320]

5.8.4 Non-fibrillar collagens

In addition to the increased synthesis and deposition of structural fibrillar collagens, cardiac fibrosis is also associated with overexpression of several non-fibrillar collagens, including collagen IV, VI, and VIII.[321,322] Fibrogenic growth factors, such as TGF-β, are responsible for induction of non-fibrillar collagens in activated fibroblasts and in fibrotic hearts. Collagen VI and collagen VIII have been implicated in fibroblast activation in experimental models of cardiac remodelling. Collagen VI promotes myofibroblast conversion,[323] and enhances fibrosis in experimental MI.[324] Collagen VIII stimulates fibroblast migration and enhances TGF-β synthesis, accentuating fibrosis in the pressure-overloaded heart.[325]

5.8.5 Matricellular proteins, galectins and proteoglycans

Matricellular proteins are a group of structurally unrelated proteins that do not play a major role in tissue homeostasis, but are up-regulated following injury and modulate cellular responses.[65] Matricellular proteins bind to structural ECM proteins and to cell surface receptors, thus transducing or modulating signalling cascades, and may also regulate activity of proteases, cytokines and growth factors. Several matricellular macromolecules, including the thrombospondins (TSPs), SPARC (secreted protein acidic and rich in cysteine), osteopontin, periostin, the tenascins-C and -X, and the members of the CCN family have been implicated in the pathogenesis of cardiac fibrosis, acting as key regulators of fibroblast activity and of immune cell function. *Table 3* summarizes the role of specific matricellular proteins in fibrotic hearts. Localized expression of matricellular proteins in areas of injury controls the spatial localization of the reparative response in infarcted hearts. On the other hand, inappropriate, excessive, or unrestrained induction of matricellular proteins may promote persistent fibrosis.

The galectins are β-galactoside-binding lectins involved in cell proliferation, differentiation, adhesion and migration that play important roles in regulation of inflammatory and immune responses.[352] Galectin-3, the best-studied member of the family, has been implicated in the pathogenesis of cardiac fibrosis. Galectin-3 is markedly and consistently up-regulated in fibrotic hearts and is predominantly expressed by activated macrophages.[353,354] Macrophage-derived galectin-3 may bind to the ECM acting as a matricellular protein, or may have cytokine-like actions. *In vitro*, galectin-3 activates fibroblasts, stimulating their proliferation, and collagen synthesis,[353] but may also promote a fibrogenic phenotype in macrophages.[355–357] Experiments on the *in vivo* role of galectin-3 in cardiac fibrosis have produced conflicting data. Some studies using galectin-3 knockout mice have suggested a critical involvement of galectin-3 in fibrosis and dysfunction of the pressure-overloaded heart,[358,359] while other investigations using very similar models reported no significant effects of galectin-3 in cardiac fibrosis and remodelling.[354,360] Due to its marked up-regulation and secretion in fibrotic cardiac conditions, galectin-3 may be a promising biomarker, and a useful predictor of adverse outcome in patients with heart failure.[361,362]

The fibrotic heart is also rich in proteoglycans, with a profile that is dependent on topography. Heparan sulfate proteoglycans (HSPGs) are typically found close to the cell surface and accumulate in the pericellular matrix. Further away from the cells, chondroitin- and dermatan sulfate-containing proteoglycans (CSPGs and DSPGs) predominate.[363] Growth factor stimulation induces expression and deposition of CSPGs in the fibrotic myocardium.[364] The cardiac ECM also contains small leucine rich proteoglycans (SLRPs), such as decorin, biglycan, fibromodulin, lumican, and osteoglycin. SLRPs have been implicated in the organization of the structural collagen-based ECM, in regulation of growth factor activation, and in signalling responses involving receptor tyrosine kinases. *In vivo* studies, has implicated several members of the SLRP family in regulation of the fibrotic response. Biglycan and osteoglycin have fibrogenic actions and contribute to the organization of the structural matrix in reparative fibrosis.[365–367] Lumican and decorin on the other hand may be anti-fibrotic, inhibiting neurohumoral and TGF-β-driven fibrogenic responses.[368,369] Fibromodulin also had anti-fibrotic effects *in vitro*; however, did not significantly affect fibrosis in an *in vivo* model of pressure overload.[370] Whether the effects of SLRPs in regulation of fibrosis are mediated through direct actions on fibroblasts, or represent effects on other cell types remains unknown.

5.9 The syndecans

The syndecans are transmembrane proteoglycans that contribute to signal transduction in growth factor-mediated fibrogenic processes. Syndecans can also undergo proteolysis, shedding their ectodomain and acting as soluble HSPGs. The bulk of the evidence from loss-of-function mouse models suggests important fibrogenic roles for syndecan-1 and -4 in myocardial diseases. Syndecan-1 is implicated in activation of reparative fibroblasts following MI, protecting the ventricle from rupture,[371] and plays a similar fibroblast-activating role in angiotensin II-induced fibrosis, promoting dysfunction.[372] Syndecan-4 has also been implicated in myofibroblast activation in infarction[373] and pressure overload models.[374] The fibrogenic actions of syndecans may be mediated through activation of TGF-β signalling cascades,[372,375] or through activation of integrin-dependent signalling.[376] Shedding of syndecans and release of soluble ectodomains with distinct properties further complicates the biological actions of syndecans.[377,378] Syndecans may mediate fibrotic conditions not only through direct actions on cardiac fibroblasts but also through effects on immune cell recruitment and function,[379,380] and on cardiomyocyte survival.[381]

5.10 CD44

The ubiquitously expressed glycoprotein CD44 is involved in regulation of a wide range of cellular functions, including cell adhesion, migration,

proliferation and differentiation.[382] In fibrotic conditions, ECM proteins such as hyaluronan, and osteopontin can bind to CD44 and transduce fibrogenic intracellular cascades. In a model of reparative fibrosis,[383] and in a model of angiotensin II-induced fibrotic cardiomyopathy,[384] CD44 transduced signals that activated cardiac fibroblasts, triggering cell proliferation and promoting activation of TGF-β cascades. Fibrogenic actions of CD44 may also be mediated through effects on immune cells.[383,385] Alternatively spliced CD44 isoforms with distinct pro- or anti-fibrotic properties contribute additional layers of complexity to the effects of this multifunctional glycoprotein.[386]

5.11 Mechanosensitive signalling pathways in cardiac fibrosis

In addition to their reparative response to injury and death of surrounding myocardial cells, which is mediated predominantly through secreted mediators, or through changes in the composition and stiffness of the ECM, fibroblasts can also become activated by mechanical stress. Fibroblasts sense mechanical forces through mechanosensitive receptors, such as integrins, ion channels, G-protein coupled receptors and growth factor receptors,[387] and activate downstream pathways that promote matrix transcription (*Figure 6*). Mechanosensitive activation of fibroblasts may have evolved to protect the integrity of the tissue, preventing catastrophic effects of mechanical forces on tissue structure. However, in the heart prolonged mechanical tension triggers persistent fibroblast activation and excessive deposition of collagens, leading to maladaptive changes in myocardial structure and functional impairment.

5.11.1 Integrins

Integrins are transmembrane receptors involved in transduction of mechanonensitive, matricellular and growth factor-mediated signals. In injured and remodelling hearts, integrins are induced and activated in fibroblasts, cardiomyocytes and immune cells, and transduce signalling cascades with crucial roles in cardiac fibrosis.[388,389] Growth factors, such as TGF-β and neurohumoral mediators, such as angiotensin II induce and activate integrins in cardiac fibroblasts.[79,232] Activated integrins interact with a wide range of binding partners and transduce downstream signalling cascades, promoting a migratory, matrix-synthetic and proliferative fibroblast phenotype. Integrins β1 and αV are the best-studied members of the family in cardiac fibrosis, and have been implicated in fibroblast activation in several different pathologic conditions. Integrin β1 is induced and activated in fibroblasts and immune cells following cardiac injury,[390] and mediates fibroblast activation in pressure-overloaded hearts.[391] In a model of angiotensin-mediated cardiac remodelling, αv integrin expressed in perivascular PDGFRβ+ cells was implicated in the development of fibrosis.[392] Whether this finding reflects conversion of PDGFRβ+ mural cells to fibroblasts is unclear. αV integrins may also exert fibrogenic actions by activating latent TGF-β.[393] Mechanosensitive integrin-mediated fibrogenic actions may involve activation of focal adhesion kinase (FAK), Ras homologue gene family/Rho-associated coiled-coil containing kinases (Rho/ROCK) and MAPK signalling.

5.11.2 FAK

FAK, a tyrosine kinase without a receptor protein, is activated in response to mechanical stress and represents a critical molecular link between mechanosensitive or growth factor-mediated integrin activation, and acquisition of a matrix-synthetic myofibroblast phenotype.[394–396] *In vivo*, pharmacologic inhibition of FAK attenuated cardiac fibrosis in a model of MI,[397,398] and FAK siRNA knockdown reduced collagen deposition in a model of pressure overload.[399] Whether these effects reflect critical FAK actions on cardiac fibroblasts remains unknown. FAK has broad effects on cardiomyocytes, vascular and interstitial cells[400,401]; thus, FAK-mediated fibrosis may be due to actions on several different cell types. Cell-specific loss-of-function approaches are needed to document the *in vivo* role of FAK in mechanosensitive or growth factor-mediated activation of fibroblasts.

5.11.3 MAPKs

In the injured and remodelling myocardium, MAPKs are activated in many different cell types by a wide range of stimuli, including mechanical stress, secreted mediators (cytokines, growth factors, and neurohumoral mediators) and matricellular proteins. Although extensive *in vitro* evidence suggests important roles for MAPK pathways in fibroblast activation, the *in vivo* role of MAPKs in fibroblast responses is less convincingly documented. p38α MAPK is the major isoform expressed in cardiac fibroblasts[402] and was found to promote myofibroblast conversion upon ischaemic injury or neurohumoral stimulation. The fibrogenic actions of p38 MAPK were attributed to actions involving the transcription factor serum response factor (SRF) and calcineurin.[114,239] In contrast, *in vivo* studies investigating fibroblast-specific actions of ERK and JNK MAPKs are lacking.

5.11.4 The RhoA/ROCK pathway

Activation of tyrosine kinases, G-protein couple receptors and integrins recruits Rho guanine nucleotide-exchange factors (RhoGEFs), leading to activation of the small GTP-binding protein RhoA. Subsequently RhoA signals through the Rho-associated coiled-coil containing kinases (ROCKs), ROCK1 and ROCK2 and plays an important role in generation of actin-myosin contractility and in regulation of cytoskeletal dynamics. The effects of ROCK on the actin cytoskeleton have profound impact on many cellular functions, including cell migration, proliferation, differentiation and survival, proliferation, migration and differentiation through actions on the actin cytoskeleton.[403] The RhoA/ROCK system may play an important role in fibrosis through effects on several different cell types, including fibroblasts, cardiomyocytes, immune cells, and vascular cells.[404] In fibrotic hearts RhoA activation may be mediated through mechanosensitive cascades or angiotensin II stimulation and has been suggested to involve p63RhoGEF.[405] The *in vivo* evidence suggesting a role for RhoA/ROCK in fibrotic remodelling of the heart is based on both pharmacologic inhibition and genetic studies. Inhibition of the RhoA-ROCK pathway reduced fibrosis of the pressure-overloaded heart.[406] Whether ROCK-mediated fibrosis reflects direct actions on fibroblasts, or indirect effects in macrophages or cardiomyocytes remains unknown. In the pressure overload model, cardiomyocyte-specific RhoA signalling triggers fibrogenic signalling through yet-unidentified paracrine effects.[407] The relative role of the ROCK isoforms in cardiac fibrosis remains poorly understood; evidence suggests a role for both ROCK1 and ROCK2 in fibroblast activation. Global hemizygous ROCK1 +/- mice and homozygous ROCK1 null animals had attenuated cardiac fibrosis in models of left ventricular pressure overload and ischaemic cardiomyopathy.[408,409] Moreover, fibroblast-specific ROCK2 was found to contribute to angiotensin II-induced fibrosis through CCN2 and Fibroblast Growth Factor (FGF)2 induction.[410]

5.11.5 Mechanosensitive ion channels

Emerging evidence suggests an important role for mechanosensitive ion channels in activation of fibroblasts and in the pathogenesis of myocardial

fibrosis. Fibrogenic mediators, such as angiotensin II and TGF-β activate members of the transient receptor potential (TRP) family of cationic channels, such as TRPC6, TRPM7, and TRPV4.[411–414] All three TRP channels have been implicated in myofibroblast conversion[411–414]; however, their *in vivo* role is not uniformly fibrogenic. TRPM7 is a channel fused to a kinase and has been reported to exert both pro- and anti-fibrotic actions. Although the bulk of the *in vitro* evidence suggests a role for TRPM7 in fibroblast activation,[413] *in vivo* the kinase domain of TRPM7 was found to protect from fibrosis, presumably through effects on macrophage inflammatory activity.[415] The potassium channel TREK1 has also been involved in fibroblast activation in pressure-overloaded hearts through effects that involve JNK activation.[416] Activation of stretch-sensitive ion channels in cardiac fibroblasts results in influx of Ca2+ and other ions. *In vitro*, angiotensin II Ca2+ oscillations have been broadly implicated in both the contractile function of myofibroblasts and in regulation of ECM synthesis[417–420] through activation of multiple signalling pathways, including MAPKs and CAMKII. However, *in vivo* evidence on the dynamics and role of Ca2+ in cardiac fibrosis is scarce.

5.11.6 YAP/TAZ

The homologous transcriptional coactivators yes-associated protein (YAP) and TAZ (transcriptional co-activator with PDZ-binding motif) are key regulators of cellular responses to mechanical stress.[421] The dynamics of the actin cytoskeleton, cadherin-mediated junctions and focal adhesions play an important role in YAP/TAZ activation. YAP/TAZ are regulated both by the Hippo pathway and through Hippo-independent mechanisms. The Hippo pathway negatively regulates YAP/TAZ through phosphorylation and activation of the large tumor suppressor (LATS)1/2 kinases. Upon activation, LATS1/2 phosphorylate YAP and TAZ to promote their nuclear export and degradation, thus abrogating their transcriptional effects. YAP/TAZ actions have profound effects on cellular phenotype, modulating cell differentiation, proliferation and survival. YAP/TAZ has been implicated in fibrotic responses in many different tissues, by accentuating TGF-β-driven activation of Smad2/3[422] and by stimulating fibroblast proliferation and synthesis of soluble fibrogenic factors.[423] In the myocardium, emerging evidence suggests that the YAP/TAZ may exert fibrogenic actions. Fibroblast-specific deletion of LATS1/2 caused fibrosis in normal hearts and accentuated matrix deposition following infarction, suggesting an important role for YAP/TAZ in activation of a secretory myofibroblast phenotype.[424] Moreover, experiments in zebrafish and in rat fibroblasts suggested that YAP/TAZ may promote activation of myofibroblasts and scar formation following MI.[425] It should be emphasized that YAP/TAZ-may regulate cardiac fibrosis, not only through direct activating effects on fibroblasts but also by promoting fibrogenic effects in cardiomyocytes,[426] or by inhibiting inflammatory activation of a fibrogenic response in epicardial cells.[427]

5.11.7 The myocardin-related transcription factor (MRTF)/SRF axis

Much like YAP/TAZ, the MRTF pathway is also regulated by cytoskeletal dynamics. Changes in the mechanical environment or growth factor signalling activate the RhoA/ROCK system and trigger F-actin polymerization, leading to MRTF translocation to the nucleus. MRTF interacts with the ubiquitously expressed transcription factor SRF, activating promoters harbouring the DNA element CArG box.[428] Interactions between SRF and members of the myocardin family of transcriptional co-activators have been implicated in stimulation of transcription of genes encoding smooth muscle cell contractile proteins. In a similar manner, in fibroblasts, the SRF/MRTF axis plays a dominant role in regulation of α-SMA transcription and subsequent myofibroblast conversion, through interactions that may involve TGF-β/Smad and RhoA/ROCK.[429–431] *In vivo* studies showed that mice with global loss of MRTF-A had attenuated fibrosis following MI and in response to angiotensin II infusion.[432] Whether these observations reflect abrogation of MRTF-dependent effects on fibroblasts remains unclear, considering that MRTF-A may also modulate cardiomyocyte and vascular cell phenotype and function.[433,434]

5.12 Oxidative stress

Mitochondrial ROS production is increased in activated fibroblasts and may critically regulate their phenotype and function, contributing to the pathogenesis of fibrotic conditions.[435] ROS are generated in ischaemic and remodelling hearts and may represent a common link between many different forms of cardiac stress and fibrosis. Several studies have suggested that the pro-fibrotic effects of fibrogenic growth factors, such as TGF-β, angiotensin II and aldosterone may be mediated through ROS generation.[436–439] TGF-β stimulation generates mitochondrial ROS[440] and also induces nicotinamide adenine dinucleotide phosphate (NADPH) oxidases that further increase ROS levels in the cells.[232,441] Increased ROS levels in activated fibroblasts stimulate ECM gene transcription, but also regulate post-translational modifications of ECM components. Both matrix-preserving and matrix-degrading effects of ROS have been reported. In addition to their effects on collagen synthesis, ROS also critically regulate synthesis and activity of MMPs involved in ECM degradation and remodelling.[442,443]

5.13 Epigenetic regulation of cardiac fibrosis

The term epigenetic regulation refers to effects of gene expression mediated through chemical modifications of the nucleosomal DNA and histone tails. In fibroblasts, post-translational modifications of histones, including acetylation and methylation, and DNA methylation have well-documented effects on expression of fibrosis-associated genes.[444–447] Histone tail lysine residues can be acetylated by histone acetyltransferases (HATs). Although non-specific pharmacologic inhibitors of HAT were reported to attenuate cardiac fibrosis in a mouse model of pressure overload,[448] robust evidence on the role of HATs in cardiac fibrotic remodelling is lacking. Acetyl groups are removed from histone tails are by a family of enzymes, the histone deacetylases (HDACs).[449] Extensive evidence derived through the use of small molecule inhibitors suggests an important fibrogenic role for HDAC activity in experimental models.[450–452] HDAC inhibition has been demonstrated to protect from diastolic dysfunction in both mouse and large animal models of cardiac ageing, hypertension, or slow progressive pressure overload.[453–455] The protective actions may be due to inhibition of fibroblast activation and proliferation, but may also involve improved myofibril relaxation in cardiomyocytes.[455,456] In addition to placement (writing) and removal (erasing) of histone modifications, a third epigenetic step involves the 'reading' of the specific histone marks. Histone acetylation creates docking sites for the 'acetyl-readers', the members of the bromodomain and extraterminal (BET) family, which recognize acetylated lysine residues through two tandem aminoterminal bromodomains.[457] BRD4 is the best studied member of the BET family in fibrotic conditions and regulates gene expression of fibrosis-associated genes in many organs.[458] In cardiac fibroblasts, BRD4 is a central regulator of the activated fibroblast

Table 4 Clinical evidence on the link between HFpEF and cardiac fibrosis

Patient population	Fibrosis marker	Finding	References
Hypertensives	Serum markers of collagen turnover (MMP2, CITP, PIIINP)	In a population of hypertensive subjects, markers of collagen turnover (circulating levels of MMP2, CITP, PIIINP) identified patients with diastolic dysfunction and HFpEF.	502
Heart failure (HFpEF and HFrEF)	Histology	In a heart failure population, histologically assessed fibrosis was comparable in HFpEF and HFrEF groups.	503
Heart failure (HFpEF and HFrEF)	Circulating biomarkers of fibrosis, CMR	HFpEF patients had elevated plasma biomarkers of inflammation and fibrosis (including TN-C, MMP3, 7 8 and GDF15) in comparison to controls. Biomarker levels were associated with CMR-assessed fibrosis (extracellular volume, ECV). However, indicators of inflammation and fibrosis were comparable between HFrEF and HFpEF patients.	504
HFpEF	CMR-assessed ECV	On the basis of ECV assessment, two subpopulations of HFpEF patients could be identified. Patients with higher ECV had a higher stiffness constant, whereas those with lower ECV had evidence of prolonged active LV relaxation.	505
HFpEF	Histology	HFpEF patients exhibited varying levels of fibrosis. Myocardial stiffness increased acutely following manoeuvres associated with transient elevations in systolic blood pressure (such as handgrip). This increase correlated with the levels of myocardial collagen deposition.	506
HFpEF with and without diabetes	Circulating biomarkers of ECM remodelling.	HFpEF patients with diabetes had increased plasma biomarkers of inflammation (IL-8 and TNFR1) and fibrosis (MMP7, TIMP1), in comparison to non-diabetic HFpEF patients.	507
HFpEF	CMR-assessed ECV	In HFpEF patients, CMR-assessed LV fibrosis was associated with impaired diastolic function.	508
HFpEF and patients at risk for HFpEF	CMR-assessed ECV	Similar levels of CMR-assessed myocardial fibrosis were present in patients with HFpEF and those 'at risk' for HFpEF (based on elevated B-type natriuretic peptide (BNP) levels). Thus, fibrosis may precede development of HFpEF.	509
Patients dying with HFpEF or conditions unrelated to heart failure	Histology	In an autopsy study, HFpEF subjects had more severe left ventricular fibrosis and capillary rarefaction than age-matched controls with non-cardiac death and no heart failure. The HFpEF group also had more severe coronary artery disease (CAD), and a higher prevalence of hypertension and diabetes that may account for the increased fibrosis. However, myocardial fibrosis was comparable in HFpEF patients with and without CAD.	510

phenotype.[459] *In vivo*, BET bromodomain inhibitors inhibited fibrosis in mouse models of pressure overload, MI,[460] and laminopathy.[461] To what extent these actions reflect direct inhibition of fibroblast activity remains unclear.

5.14 Non-coding RNAs in cardiac fibrosis

A large and growing body of evidence suggests that noncoding RNA transcripts, including microRNAs (miRNAs) and long non coding RNAs (lncRNAs), are involved in the pathogenesis of cardiac fibrosis by regulating gene expression at the post-transcriptional level.[462,463] miRNAs have been suggested to target several crucial fibrogenic cascades, including TGF-β/Smad, angiotensin II/MAPK, RhoA/ROCK, MRTF/SRF, and the cationic ion channels.[464] It has been suggested that in some cases fibroblast-derived miRNAs may also mediate paracrine effects through their secretion in the cardiac interstitium as exosome cargo, and subsequent uptake by other cell types, such as cardiomyocytes and immune cells.[465] Understanding of the role of miRNAs in fibrotic conditions is hampered by the complexity of their actions in many different cell types, and by the broad range of their molecular targets. In a study examining the *in vitro* effects of 194 different miRNAs, differentially expressed in inflamed hearts, on cellular function, a large percentage of the corresponding mimics (25.8%) modulated fibroblast function.[466] Effects of the mimics on other cell types (including cardiomyocytes and macrophages) highlighted the need for *in vivo* studies in order to determine the role of miRNAs in the fibrotic response. *In vivo* data have suggested that several miRNAs, including miR-15, miR-29, miR-101, and miR-1954, may be involved in negative regulation of cardiac fibrosis through inhibitory effects on pathways activated by fibrogenic growth factors or matricellular proteins.[467–470] MiR-21, miR671-5p, and miR-144-3p on the other hand, have been suggested to exert fibrogenic actions.[471–473] The basis for this remarkable complexity in miRNA-mediated regulation of fibrogenic processes remains unclear.

An emerging body of evidence suggests than lncRNAs represent an additional layer of complexity in the regulation of cardiac fibrosis,

Table 5 The prognostic implications of fibrosis in HFpEF patients

Fibrosis-associated marker	Findings	References
Histology	Fibrosis predicted adverse outcome in HFrEF patients, but NOT in HFpEF patients.	503
Serum galectin-3	In a HFpEF population, galectin-3 levels were modestly elevated, were inversely associated with functional performance, and were independently associated with adverse outcome.	511
Molli-ECV	In HFpEF patients MOLLI-ECV (CMR-assessed) correlated with histological evidence of fibrosis (assessed through trichrome staining in a smaller number of patients). However, ECV-associated fibrosis was not independently associated with adverse outcome.	512
Serum biomarkers of collagen turnover	In a HFpEF patient population, circulating levels of collagen turnover biomarkers and osteopontin levels were not independently associated with outcome (mortality and hospitalizations), despite association with single-variable analysis.	513
Serum TSP-2	Serum TSP-2 levels predicted adverse outcome in HFpEF patients.	514
Multiple serum biomarkers, including markers of ECM remodelling.	A multimarker approach combining biomarkers reflecting calcification, inflammation, metabolism, angiogenesis and matrix turnover predicted outcome in a HFpEF population.	515
LGE (late gadolinium enhanced)-MRI	In a HFpEF population, myocardial fibrosis assessed through LGE-MRI was associated with adverse outcome.	516
CMR	In HFpEF patients, CMR-assessed fibrosis was associated with worse outcome.	517
CMR	Myocardial fibrosis was associated with worse outcome in both HFpEF patients and in subjects 'at risk' for HFpEF (based on high BNP levels). HFpEF patients without fibrosis had better outcome.	509
CMR	Myocardial fibrosis assessed through CMR-based extracellular volume assessment was increased in HFpEF patients and was an independent predictor of outcome.	518

exerting broad effects on fibroblast phenotype and function.[474] Maternally expressed gene 3 (*Meg3*),[475] Wisp2 super-enhancer associated RNA (*Wisper*),[476] pro-cardiac fibrotic lncRNA,[477] and plasmacytoma variant translocation 1 (PVT1) have been suggested to exert fibrogenic actions on the myocardium. Much like miRNAs, lncRNAs may also be implicated in paracrine actions that regulate fibrosis. Hypoxic and stressed cardiomyocytes were found to secrete vesicles containing fibrogenic lncRNAs, such as Neat1, that may promote fibroblast activation.[478] Whether lncRNAs are involved in the pathogenesis of human conditions associated with cardiac fibrosis remains unknown; however, associative studies have shown a correlation between expression of lncRNAs and ECM genes in ischaemic cardiomyopathy patients.[479]

5.15 Endogenous mechanisms involved in negative regulation of cardiac fibrosis

Endogenous signals restraining fibroblast expansion and inhibiting fibroblast function have evolved to prevent prolonged, excessive or expanding fibrogenic responses in healing tissues. In the infarcted heart, scar maturation is associated with apoptosis of significant numbers of myofibroblasts.[480] Many other fibroblast-like cells survive[481] but become quiescent and may reduce their levels of expression of ECM proteins. Deactivation of infarct myofibroblasts may involve in part passive removal of fibrogenic mediators, such as clearance of matricellular proteins and growth factors, but may also require active processes and induction of anti-fibrotic signals. Perturbations of these anti-fibrotic pathways may be involved in the pathogenesis of fibrosis following injury. Unfortunately, our understanding of the specific molecular signals involved in negative regulation of fibrosis remains limited, and their involvement in human myocardial diseases is uncertain. TGF-β is a major target of anti-fibrotic signals and TGF-β signalling cascades are tightly regulated by endogenous inhibitory mediators that act at the receptor level, or by inhibiting downstream intracellular cascades.[482] In the failing heart, release of a cleaved soluble form of the accessory TGF-β receptor endoglin was found to act as a negative regulator of TGF-β signalling.[483] In the pressure-overloaded heart, expression of the TGF-β pseudo-receptor BAMBI (Bone Morphogenetic Protein/BMP and activin membrane-bound inhibitor) has been suggested to downmodulate TGF-β signalling, attenuating its profibrotic actions,[484] and serving as a molecular sink for TGF-β. Induction of the inhibitory Smads, such as Smad7 and Smad6, may restrain pro-fibrotic TGF-β responses through interactions with the TGF-β receptors, or the receptor-activated Smads.[485] Other anti-fibrotic signals may inhibit responses to neurohumoral activators of fibrosis. Apelin signalling has been proposed as a negative regulator of angiotensin II-mediated fibrotic remodelling.[486] Moreover, fibroblasts may modulate their response to the fibrogenic actions of angiotensin II by altering their relative expression of the pro-fibrotic AT1 receptor vs. the anti-fibrotic AT2.[83,84] Activation of a p53- and p16-mediated senescence programme in cardiac fibroblasts may also be implicated in negative regulation of cardiac fibrosis.[487] As previously discussed, up-regulation of anti-fibrotic miRNAs may serve an important inhibitory mechanism, preventing excessive fibroblast activation in remodelling hearts.[467–469] Whether such inhibitory signals are selectively induced in subsets of suppressive interstitial cells with an anti-fibrotic phenotype remains unknown.

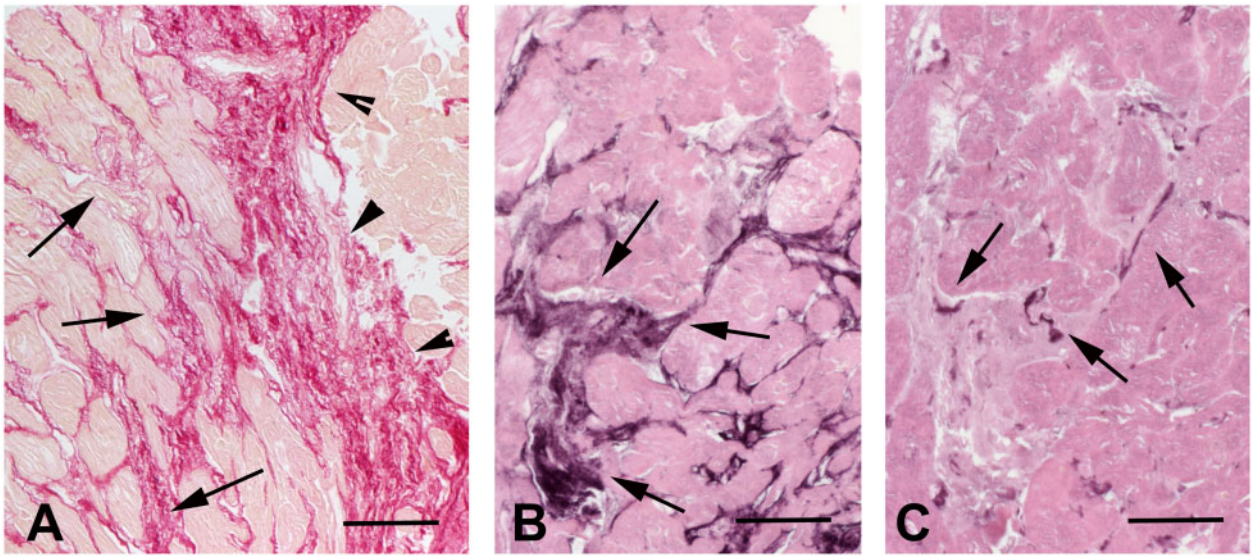

Figure 7 Fibrotic changes in patients with ischaemic cardiomyopathy in the absence of myocardial infarction. Patients with ischaemic cardiomyopathy undergoing aortocoronary bypass surgery underwent transesophageal echocardiography (TEE)-guided biopsy to sample non-infarcted segments perfused by partially occluded coronaries. (*A*) Prominent interstitial fibrosis (arrows), and occasional small foci of replacement fibrosis (arrowheads) were typically noted in these segments. (*B*) Immunohistochemical staining shows interstitial deposition of the matricellular protein tenascin-C (arrows), an indicator of active remodelling. C. α-SMA staining identifies myofibroblasts in the cardiac interstitium (arrows). The findings of the study (originally published in ref.[28] and ref.[27]) suggested that segments with an active interstitum, characterized by more inflammatory cells, more myofibroblasts, but less mature collagen had a higher chance of recovery following revascularization. These observations may support a role for interstitial cells in resolution and regression of fibrosis. Scalebar = 50 μm.

6 Fibrosis in human myocardial diseases

In most clinical studies and in a wide range of myocardial pathologies, the extent of cardiac fibrosis predicts adverse outcome.[488] However, this does not necessarily imply causation. Depending on the underlying disease and the pathophysiologic context, the presence of cardiac fibrosis may contribute to dysfunction and adverse outcome, or simply reflect activation of a reparative process.

6.1 Fibrosis in myocardial infarction

MI is typically caused by rapid cessation of coronary blood flow, due to rupture of an atherosclerotic plaque. Occlusion of the vessel causes prolonged ischaemia and activates a 'wavefront' of cardiomyocyte death extending from the more susceptible subendocardium to the subepicardium.[489] Because the adult mammalian heart has negligible regenerative capacity, massive cardiomyocyte necrosis triggers an inflammation-driven reparative fibrotic response that leads to formation of a scar.[490] Thus, infarctive fibrosis is reparative; formation of a scar following infarction is crucial for protection of the ventricle from catastrophic mechanical complications, such as cardiac rupture. Clearly, patients with larger scars have worse prognosis.[491,492] However, this association likely reflects the relation between more extensive loss of functional cardiomyocytes and poor outcome, rather than the adverse consequences of a primary fibrotic process.

Although a collagen-based scar cannot replace the rhythmic contractile function of healthy cardiomyocytes, the biochemical composition of the infarct is a major determinant of cardiac mechanics and affects outcome. Decades of experimental work have suggested that inflammation and fibrosis following infarction need to be tightly regulated in order to protect the ventricle from catastrophic structural failure, while limiting the development of heart failure. Formation of myofibroblast arrays that form an organized network of collagen fibres is crucial to prevent cardiac rupture.[232] Timely activation and suppression of inflammatory and pro-fibrotic signals, and spatial containment of the reparative process within the area of injury are critical to protect from adverse remodelling and dysfunction. Prolonged, unrestrained or excessive activation of fibroblast-driven matrix deposition can contribute to the development of post-infarction heart failure.[493] Overactive inflammatory signalling may promote protease activation, causing ECM degradation, reduction of the tensile strength of the scar and ventricular dilation.[494] On the other hand, overactive matrix-synthetic pathways may increase stiffness promoting diastolic dysfunction.

Identification of associations between scar composition and prognosis in patients with MI poses major challenges, considering the limitations of current imaging technologies and biomarker-based approaches. Some studies have suggested associations between cardiac magnetic resonance (CMR)-derived scar composition parameters and clinical outcome in patients surviving MI. Magnetic resonance measurements reflecting the texture of the scar were associated with an increased incidence of arrhythmias following infarction.[495] Moreover, high 'entropy' within the scar (an indicator of inhomogeneous scar composition) was associated with mortality and increased arrhythmogenicity.[496] The potential relation between these crude imaging-derived parameters and specific cellular or biochemical changes remains unknown.

As the scar matures, infarct fibroblasts lose their myofibroblast characteristics[30,481] and may undergo apoptosis.[480] However, in large

infarcts, pressure and volume overload due to the massive loss of contractile cells may result in progressive interstitial fibrosis in the border zone and in the remote remodelling myocardium.[497] Associative evidence in experimental models suggests that these interstitial fibrotic changes may contribute to systolic and diastolic dysfunction leading to post-infarction heart failure. In animal models, the beneficial effects of ACE inhibition and AT1 receptor blockade following infarction were associated with attenuated interstitial collagen accumulation in non-infarcted myocardial segments.[498] However, data on the extent and significance of fibrotic remodelling of non-infarcted segments in patients with MI are conflicting. In a biochemical study using explanted hearts and autopsy material, no significant increases in collagen content were noted in non-infarcted myocardium remote from the scar.[499] In contrast, in patients with reperfused ST-elevation MI, CMR showed expansion of the extracellular volume in non-infarcted segments that was associated with progression of contractile dysfunction.[500]

6.2 Fibrosis in chronic heart failure

Myocardial fibrotic remodelling is found in many pathologic conditions associated with heart failure. In many cases, it is unclear whether fibrosis represents the main causative cellular process responsible for dysfunction, or simply reflects a reparative response to a primary injurious insult. However, in most conditions, the presence and severity of myocardial fibrosis carries prognostic information and predicts adverse outcome. Prominent fibrosis is found in patients with both heart failure with reduced ejection fraction (HFrEF) and HFpEF and is associated with all major pathophysiologic conditions that cause heart failure, including chronic ischaemia, genetic cardiomyopathies, pressure and volume overload, and metabolic diseases.

6.3 Cardiac fibrosis and HFpEF

Nearly half of heart failure patients have a preserved ejection fraction. HFpEF patients tend to be older, have a high prevalence of hypertension, obesity and diabetes, and typically exhibit reduced ventricular and vascular compliance. Considering the close association between fibrosis and tissue stiffness (both myocardial and vascular), it is tempting to hypothesize that deposition of cross-linked collagen in the cardiac interstitium or in the vascular wall may promote diastolic dysfunction and play a prominent role in the pathogenesis of HFpEF.[501] A large body of evidence from human patients suggests that at least a subset of HFpEF patients exhibits cardiac fibrosis (*Table 4*). In some studies, the association between HFpEF and fibrosis was documented through histological analysis of biopsied or autopsied myocardial samples[503,510]; however, in most studies CMR-based measurements of extracellular volume was used to assess fibrotic changes. HFpEF patients with evidence of fibrosis tend to have a higher stiffness constant in comparison to those without significant fibrosis[505] and exhibit acute increases in myocardial stiffness with transient elevations of blood pressure.[506] Moreover, some investigations have suggested that fibrosis may precede the development of HFpEF in high-risk patients, thus supporting a possible causative relation.[509]

Extensive evidence suggests that fibrosis in HFpEF has prognostic implications (*Table 5*), indicating that HFpEF patients without fibrosis have a more favourable outcome.[509] It should be emphasized that in practically all studies suggesting prognostic implications, fibrosis was measured using CMR or circulating serum biomarkers. In contrast, a study using histological analysis failed to reveal a relation between fibrosis and prognosis in HFpEF patients.[503] The lack of histological evidence to support the prognostic role of fibrosis in HFpEF may not necessarily reflect the absence of a relation, but may reflect the challenges in performing adequately powered biopsy studies in HFpEF patients.

6.4 Cardiac fibrosis and HFrEF

Fibrotic changes are typically observed in patients with HFrEF regardless of the underlying aetiology. Extensive evidence suggests that the severity of fibrosis in HFrEF patients is associated with worse outcome. In heart failure patients, myocardial fibrosis quantified by CMR was associated with subsequent hospitalizations and mortality across the spectrum of ejection fraction and heart failure stage.[519] Moreover, in new onset HFrEF, the absence of fibrosis was associated with favourable prognosis.[520] Although the effects of fibrosis on myocardial stiffness and diastolic function are intuitive, causative links between fibrotic interstitial changes and systolic dysfunction are less convincingly documented. Perturbations of the interstitial matrix network may depress systolic function through several distinct mechanisms. First, expansion of the interstitial matrix may disrupt excitation-contraction coupling. Second, certain forms of fibrosis may be associated with prominent activation of proteases, leading to disruption of key molecular links between the matrix and the sarcomeric contractile apparatus, and leading to systolic dysfunction.[521] Generation of matrix fragments in protease-rich fibrotic lesions may stimulate pro-inflammatory signalling, further exacerbating systolic dysfunction.[234] Third, perturbations in the native matrix network may deprive cardiomyocytes from crucial matrix-dependent molecular signals that promote cardiomyocyte survival, resulting in a form of cell death termed 'anoikis'.[522] Fourth, development of perivascular fibrosis may result in microvascular dysfunction and reduce perfusion of cardiomyocytes under conditions of stress, leading to depression of systolic function.

6.5 Cardiac fibrosis in chronic ischaemic cardiomyopathy

Patients with ischaemic cardiomyopathy often have evidence of replacement fibrosis, even in the absence of a history of MI, reflecting in many cases silent coronary events. However, both experimental and clinical studies suggest that episodic demand ischaemia can cause fibrosis, in the absence of infarction. In a subset of patients dying with severe coronary artery disease, severe interstitial and perivascular fibrosis was found in the absence of grossly visible acute or healed MI.[523] Moreover, in patients with ischaemic cardiomyopathy undergoing aortocoronary bypass surgery, segments without evidence of prior MI, but perfused through a partially occluded coronary, exhibited interstitial fibrosis, associated with contractile dysfunction[27,28] (*Figure 7*). The characteristics of the fibrotic lesions predicted recovery of function following revascularization. Segments with a highly cellular interstitium, and more abundant myofibroblasts and inflammatory cells were more likely to recover after revascularization; in contrast segments with hypocellular interstitial fibrosis and increased collagen deposition had persistent dysfunction. In a mouse model, brief repetitive myocardial ischaemia and reperfusion-induced fibrosis in the absence of infarction, by activating an ROS-dependent chemokine-mediated fibrogenic programme.[39,129] Thus, brief ischaemic insults, or low perfusion states associated with episodic demand ischaemia may be sufficient to stimulate interstitial myocardial fibrosis, contributing to the pathogenesis of ischaemic cardiomyopathy.

6.6 Fibrosis in genetic cardiomyopathies

Although extensive associative data suggest that fibrosis often accompanies cardiomyocyte changes in patients with genetic cardiomyopathies,

the role of fibrotic remodelling in mediating dysfunction and disease progression is unclear. Limited data from animal model studies using cell-specific loss-of-function approaches support the notion that fibroblasts in certain genetic cardiomyopathies do not simply respond to injury in a reparative manner, but may undergo activation, secreting large amounts of ECM proteins and accentuating dysfunction. In sarcomere mutation carriers, elevations of circulating biomarkers of collagen synthesis were noted prior to development of overt hypertrophic cardiomyopathy (HCM),[524] suggesting that fibrogenic activation may precede the clinical manifestations. In transgenic mice with cardiomyocyte-specific overexpression of a truncated cardiac myosin binding protein C (a mouse model that recapitulates features of a relatively common human form of HCM), abrogation of TGF-β signalling specifically in myofibroblasts attenuated fibrosis, reduced cardiomyocyte hypertrophy, and prolonged lifespan.[525] Some genetic forms of dilated cardiomyopathy (DCM) are also associated with severe and diffuse fibrosis,[526] and in mouse models of genetic DCM, fibrogenic activation occurs early and has been suggested to contribute to disease progression.[527,528] Moreover, in patients with arrhythmogenic right ventricular cardiomyopathy (ARVC), mutations in genes encoding desmosome proteins trigger expansion and activation of fibro-adipocytes, leading to collagen deposition and fatty infiltration[529] and may also activate a fibrogenic programme in cardiomyocytes.[530,531]

Whether certain genetic conditions can cause cardiomyopathy in human patients through primary activation of fibroblasts is unknown. Patients with mutations in desmoplakin, the primary force transducer between cardiac desmosomes and intermediate filaments, exhibited a cardiomyopathy characterized by early onset of left ventricular fibrosis that preceded systolic dysfunction and was associated with arrhythmias.[532] In autopsies of young patients with sudden cardiac death, cardiac fibrosis is often found in the absence of any identifiable cardiomyocyte pathology.[533] In many of these patients mutations in cardiomyocyte-specific genes are identified,[534] supporting the notion that fibrotic remodelling involves paracrine cardiomyocyte-derived signals, and not primary activation of interstitial cells.

6.7 Fibrosis in heart failure associated with pressure overload

Left ventricular pressure overload induces interstitial and perivascular fibrosis in several common pathologic conditions, including hypertensive heart disease and in the cardiomyopathy associated with aortic stenosis. Long-standing hypertension triggers collagen deposition in the interstitium and in perivascular areas, increasing myocardial stiffness and contributing to the pathogenesis of HFpEF.[535,536] Moreover, in patients with aortic stenosis, fibrosis assessed through CMR was more extensive in women,[537] and was independently associated with mortality.[538–540] Although concomitant ischaemic heart disease may be responsible for fibrosis in aortic stenosis patients, in many cases, significant fibrotic lesions are noted in the absence of coronary atherosclerosis,[541] reflecting the consequences of chronic pressure overload.

In animal models, left ventricular pressure overload induces early hypertrophy, fibrosis and diastolic dysfunction, followed by decompensation, chamber dilation, and development of systolic dysfunction.[542,543] Although the severity of fibrotic changes and the time course of cellular events are dependent on the specific characteristics of the pressure overload model, the alterations recapitulate many of the geometric and functional changes observed in patients with untreated malignant hypertension, or uncorrected critical aortic stenosis. Several mechanisms cooperate to activate fibroblasts in the pressure overloaded heart. First neurohumoral activation plays an essential role in promoting myofibroblast conversion and acquisition of a matrix-synthetic programme.[544] Second, induction of matricellular proteins locally activates growth factor-mediated signalling in fibroblasts, stimulating ECM protein synthesis.[65,545] Third, high intracardiac pressures directly activate mechanosensitive cascades, triggering fibrogenic signalling in cardiac fibroblasts.[546] Fourth, inflammatory cytokines and growth factors released by immune cells, or by stressed cardiomyocytes may significantly contribute to fibroblast activation. The critical contribution of lymphocytes and macrophages in the pathogenesis of fibrosis and dysfunction following pressure overload has been documented by several experimental studies.[157,547,548] Activation of immune cells in the pressure-overloaded myocardium occurs in the absence of significant cardiomyocyte necrosis and may be mediated through stimulation of neurohumoral pathways, generation of matrix fragments, or ROS-dependent pro-inflammatory signalling.

6.8 Fibrosis in conditions associated with volume overload

Left ventricular volume overload is the dominant pathophysiologic perturbation in patients with severe valvular regurgitant lesions. In patients with severe aortic regurgitation fibrotic remodelling has been extensively documented.[549–551] Animal models and *in vitro* experiments have provided interesting insights into the unique effects of volume overload on fibroblast function and ECM remodelling. In contrast to the net collagen deposition typically observed in pressure-overloaded hearts, volume overload was found to cause a marked loss of interstitial collagen,[552] associated with MMP induction.[552,553] Moreover, it has been suggested that volume overload induces fibronectin synthesis in cardiac fibroblasts without significantly affecting collagen production.[554] Activation of a matrix-degrading environment may explain the pronounced chamber dilation observed in volume-overloaded hearts. The molecular basis for predominant activation of MMPs in volume overloaded hearts remains unknown. Lesions causing volume overload likely generate a unique mechanical environment with distinct effects on cardiomyocytes and interstitial cells. However, the network of molecular cascades activated by volume overload-induced mechanical stress has not been characterized.

6.9 Fibrosis in obesity and diabetes

Patients with diabetes and severe obesity develop fibrosis that is not limited to the myocardium, but may also affect other organs, such as the kidney. The basis for accentuated fibroblast activation in diabetic patients remains unclear. Animal model studies have suggested that expansion of the cardiac interstitium and collagen deposition in obese diabetic mice does not require myofibroblast conversion, but is associated with activation of a matrix-synthetic programme in cardiac fibroblasts.[44] Hyperglycaemia has direct activating effects on cardiac fibroblasts, stimulating fibrogenic TGF-β cascades,[555] leading to accumulation of advanced glycation end-products (AGEs) that crosslink the cardiac ECM, transducing ROS-dependent fibrogenic signals, and triggering receptor for AGE-mediated fibroblast activation.[213,556–558] Glucose-independent pathways may also stimulate fibroblast activation through the effects of adipokines, neurohumoral pathways, or ET-1 signalling.[557] Microvascular inflammation is also induced and may play an important role in fibroblast activation in diabetics.[559,560] Moreover, metabolic dysregulation in diabetic subjects has been found to induce matricellular proteins with a prominent role in activation of fibrogenic cascades, such as TSP-1.[326,561]

In human patients, obesity and diabetes are associated with fibrotic cardiomyopathic changes[562] and with a marked increase in the risk of HFpEF, that is independent of the presence of ischaemic heart disease.[563,564] Changes in the interstitial ECM may contribute to the distinct obesity-related phenotype of human HFpEF,[565] which is also associated with increased plasma volume, increased hypertrophy and accentuated right ventricular dilatation, despite lower plasma levels of natriuretic peptides.[565,566]

6.10 Fibrosis in the ageing heart

Ageing is typically associated with diastolic dysfunction and preserved systolic contractility, thus contributing to the pathogenesis of HFpEF. In both human patients and in experimental models, ageing is associated with interstitial deposition of ECM proteins in both ventricles and atria.[567] The cell biological basis of ageing-related fibrosis involves expansion of resident fibroblasts of epicardial origin that infiltrate the cardiac interstitium.[568] Several activating signals may promote fibroblast proliferation and activation in the ageing heart, including generation of ROS, neurohumoral activation, TGF-β, and inflammatory chemokines and cytokines.[569,570] Single-cell transcriptomics in mouse models demonstrated that ageing is associated with alterations in cardiac fibroblast phenotype, characterized by increased expression of osteogenesis-associated genes, impaired angiogenic activation of endothelial cells, and a pro-inflammatory phenotype.[571] To what extent, ageing-associated fibrotic changes contribute to the functional perurbations in senescent hearts remains unclear. In senescent mice, exercise training improved diastolic function without affecting cardiac fibrosis,[572] highlighting the importance of fibrosis-independent processes in the cardiomyopathy associated with ageing. It should be emphasized that, although senescent mice have higher baseline myocardial collagen levels, they also exhibit an impaired fibroblast response following injury. In a model of reperfused infarction, senescent mouse hearts had impaired healing, reduced deposition of collagen in the scar and accentuated cardiac dilation.[573] Perturbations in reparative fibroblast functions in senescent mice may involve impaired responses to fibrogenic growth factors.[573]

6.11 Fibrosis in dysrhythmias

In addition to its effects on cardiac function, fibrosis may perturb propagation of the electrical impulse, and may generate re-entry circuits, thus contributing to the pathogenesis of dysrhythmias.[574,575] Links between fibrosis and increased risk for ventricular arrhythmias have been documented in practically every cardiac pathologic condition using CMR for assessment of fibrotic remodelling[576] Fibrosis was found to predict ventricular arrhythmias and/or sudden cardiac death in patients with ischaemic and dilated cardiomyopathy,[577] HCM,[578] mitral valve prolapse,[579] and myocarditis.[580] The relation between specific cellular and biochemical characteristics of the fibrotic lesions and the susceptibility to arrhythmias remains poorly understood. It is likely that the biochemical profile of the interstitial ECM, as well as the composition of the interstitial cellular infiltrate may be important determinants of arrhythmogenic potential in fibrotic hearts. Animal model investigations suggest that infarct myofibroblasts may be electrically coupled with border zone cardiomyocytes; albeit with slower voltage responses, reflecting less effective conduction across heterocellular junctions.[581,582] α-SMA expression and TGF-β stimulation have been suggested to increase fibroblast arrhythmogenic actions in the injured heart.[583,584] Because even small changes in conduction and impulse propagation may greatly impact susceptibility to ventricular arrhythmias, it has been suggested that manipulation of non-excitable fibroblasts in the infarct may have profound effects on the arrhythmogenic process.[585] On the other hand, deposition of ECM in fibrotic regions with a low cellular content may be associated with marked disruption of impulse propagation.

The arrhythmogenic effects of cardiac fibrosis are not limited to the left ventricular myocardium. In patients with pulmonary hypertension, right ventricular fibrosis may not only perturb function, but also contribute to the pathogenesis of arrhythmias.[586,587] In patients with ARVC, prominent fibrosis is typically associated with a high susceptibility to ventricular arrhythmias.[588,589]

On the other hand, fibrotic atrial remodelling is often associated with elevated filling pressures in patients with heart failure and predisposes to initiation and maintenance of atrial fibrillation.[590–594] The severity of atrial fibrosis has also been linked to the effectiveness of treatment interventions for atrial fibrillation: more severe fibrotic changes reduce the chances of success.[595] A large body of evidence suggests that atrial fibrosis can be useful for risk stratification in patients with atrial fibrillation, serving as a predictor of recurrence, persistence, heart failure and stroke.[596] The relation between the biochemical characteristics of the fibrotic response and the susceptibility to atrial fibrillation has not been systematically studied. It has been suggested that, although early and evolving fibrotic lesions may contribute to the pathogenesis of atrial fibrillation, very extensive fibrosis may decrease atrial excitability, reducing atrial fibrillation burden.[597] Data supporting this interesting hypothesis are lacking.

7. Targeting the fibrotic response in myocardial disease

Anti-fibrotic strategies have been proposed as promising therapeutic approaches for patients with myocardial diseases. Unfortunately, the rationale for implementation of such approaches is often based on oversimplified concepts and a misguided view of 'cardiac fibrosis' as a single disease entity that resembles primary fibrotic conditions in other systems, such as idiopathic pulmonary fibrosis or scleroderma. It should be emphasized that cardiac fibrosis is not a specific disease, but rather a common pathologic abnormality that accompanies most myocardial diseases, and often represents an appropriate secondary reparative response to a primary insult. Because the adult mammalian heart has negligible regenerative potential, myocardial insults causing cardiomyocyte necrosis are repaired through generation of fibrous tissue. Even in conditions associated with predominant interstitial and perivascular fibrosis, in the absence of significant cardiomyocyte death (such as hypertensive heart disease, or the cardiomyopathy associated with diabetes and obesity), the relative contribution of fibrosis in dysfunction and adverse outcome is unclear. Although in many cases, fibrotic changes are likely contributors to functional impairment and arrhythmogenesis, there is no human myocardial disease with a well-documented primary fibrotic cause. Thus, recommendations regarding anti-fibrotic interventions should avoid generalizations and simplistic views, while taking into account the pathophysiologic heterogeneity of myocardial disease and the distinct profile of fibrotic changes found in each type of myocardial injury.

7.1 Can we reverse myocardial fibrosis?

A key question that needs to be answered when designing anti-fibrotic strategies for patients with myocardial disease is whether established cardiac fibrosis can be reversed, in the absence of an effective

regenerative intervention. The answer is currently based more on intuition and clinical experience, than on experimental evidence. Reversibility is likely dependent on the aetiology of fibrosis, the extent of fibrotic remodelling, and the biochemical properties of the ECM, which are typically dependent on the age of the lesions. Predicting reversal of fibrosis seems straight-forward for lesions in the extremes of the pathophysiologic spectrum. For example, established replacement fibrosis due to a large MI typically would require major advances in myocardial regeneration, a visionary goal of modern cardiovascular research. On the other hand, early evolving interstitial or perivascular fibrotic changes are more likely to be reversible. In an experimental mouse model of ischaemic interstitial fibrosis due to brief repetitive ischaemia and reperfusion in the absence of MI, discontinuation of the ischaemic insults after 7 or 28 days resulted in reversal of fibrosis.[39] Moreover, pharmacologic treatment with ACE inhibitors in experimental models reversed hypertensive fibrosis.[598] Clinical studies have generated inconclusive and, in some cases, conflicting data, reflecting the heterogeneity of fibrotic lesions. In a small population of hypertensive patients with left ventricular hypertrophy, a 6-month course of lisinopril reduced fibrosis and attenuated diastolic dysfunction.[599] Moreover, in patients with aortic stenosis, imaging studies showed a significant reduction in matrix volume, accompanied by a proportionally greater reduction of cell volume, but consistent with the possibility of regression of diffuse fibrotic lesions.[600,601] In contrast, in patients with advanced end-stage heart failure, aggressive unloading through a left ventricular assist device did not affect the extent of cardiac fibrosis[602] and in patients with severe aortic stenosis, valve replacement did not reverse established fibrotic lesions.[603] The conflicting findings likely reflect distinct responses of various types of fibrotic lesions, but also the methodology used to assess reversal and the timing of assessment after the unloading procedure.

The cellular basis for reversal of cardiac fibrotic lesions remains enigmatic. Clearance of collagen and other matrix proteins from the fibrotic heart would be expected to require release and activation of proteases from specialized populations of anti-fibrotic interstitial cells. However, whether specific subsets of 'fibrosis-resolving' immune cells or fibroblasts are implicated in regression of fibrotic lesions remains unknown. In a group of patients with ischaemic cardiomyopathy undergoing aorto-coronary bypass surgery, recovery of function after revascularization was more likely in myocardial segments with a higher cellular content, increased numbers of macrophages, higher levels of matricellular proteins, more myofibroblasts and lower collagen content.[27,28] Although improved dysfunction does not necessarily imply reduced fibrosis, such observations may suggest that subpopulations of macrophages and fibroblasts may be required for resorption and resolution of fibrotic lesions.

7.2 Strategies for inhibition of cardiac fibrosis in chronic heart failure

Independently of our capability to reverse established fibrotic lesions, inhibition of active fibrotic responses may benefit subsets of patients with chronic heart failure. Based on the abundant experimental evidence implicating specific cytokines, growth factors, matricellular proteins, integrins and mechanosensitive signalling pathways in activation of cardiac fibroblasts, it is not surprising that a vast range of 'anti-fibrotic' pharmacologic approaches has been proposed as promising therapy for heart failure patients. Some established heart failure therapies targeting neurohumoral pathways (such as ACE inhibition, AT1 receptor blockade, mineralocorticoid antagonism, and β-adrenergic blockade) may reduce mortality and event rates in heart failure patients, at least in part through inhibition of fibrosis. Experimental studies have suggested that several additional anti-fibrotic interventions may hold therapeutic promise, including inhibition of fibrogenic pro-inflammatory cytokines (such as IL-1 and CCL2),[129,604,605] anti-TGF-β approaches, αV or β1integrin inhibition, strategies inhibiting ECM cross-linking enzymes,[316] and blockade of common kinase pathways. Recently, a fibroblast ablation strategy using adoptive transfer of CD8+ T cells engineered to expressed a chimeric antigen receptor against fibroblast activating protein reduced fibrosis and improved dysfunction in a mouse model of cardiac remodelling induced through neurohumoral activation.[606] Unfortunately, the path towards clinical translation for anti-fibrotic approaches in myocardial disease is long and uncertain, as therapeutic implementation is hampered by several major challenges.

First, most of these approaches would require very early therapeutic intervention at a pre-clinical stage. In animal models, these interventions are typically initiated at an early stage of a dynamic model of rapidly progressive fibrotic disease. This is impractical in heart failure patients who are likely to present with more advanced fibrotic lesions.

Second, many of these pathways have important protective or reparative functions in response to a variety of injurious stimuli. Thus, chronic targeting of these pathways over the multi-year period typically required for progression of myocardial fibrosis may not be safe.

Third, the reparative nature of cardiac fibroblast activation and the pathophysiologic heterogeneity of human myocardial conditions associated with fibrosis greatly complicate implementation of anti-fibrotic strategies. We currently do not know to what extent myocardial dysfunction is caused by fibrosis. In many patients, fibrotic myocardial responses reflect appropriate activation of reparative or protective mechanisms. Abrogation of these responses may carry significant risks.

7.3 The need for pathophysiologic stratification of patients with fibrotic changes

In contrast to the well-defined injurious role of fibrogenic signalling in primary fibrotic conditions, such as progressive systemic sclerosis or idiopathic pulmonary fibrosis, the contribution of fibrotic remodelling to dysfunction, arrhythmogenesis and adverse outcome in patients with myocardial disease is uncertain and context-dependent. Thus, there is a need for pathophysiologic stratification of heart failure patients, in order to identify subpopulations with inappropriate, progressive or unrestrained fibrogenic responses that may be candidates for anti-fibrotic interventions.

Considering the potential risks of chronic anti-fibrotic therapies, targeting fibrogenic activation may be most attractive for patients with dynamic, rapidly evolving disease processes, such as MI, that could benefit from a brief course of therapy. The combined use of biomarkers reflecting inflammatory activation and ECM metabolism,[607,608] or imaging studies[609] may identify patient subpopulations with excessive and progressive ECM deposition or degradation that may benefit from modulatory therapy. Patients with prominent and inappropriate ECM deposition (that is typically associated with increased ventricular stiffness and diastolic dysfunction) may benefit from brief inhibition of fibrogenic growth factors. On the other hand, patients with accentuated and prolonged inflammatory activation typically exhibit enhanced protease activation leading to dilative remodelling and systolic dysfunction. These patients may be candidates for targeted anti-inflammatory therapy, such as IL-1 inhibition, or CCL2 antagonism.[493,610]

8. Conclusions

The pathophysiologic heterogeneity of fibrotic myocardial conditions and the complexity of fibroblast responses to injury greatly complicate development of anti-fibrotic strategies in myocardial disease. Despite the clear association between the severity of cardiac fibrosis and adverse outcome in patients with heart disease, documentation of causative relations remains challenging. Fibroblast activation is a critical reparative response following myocardial injury and may preserve structure and function not only in MI but also in chronic heart failure. Progress in the field requires new knowledge in several different directions. First, considering the heterogeneity of interstitial cells, characterization of fibroblast subsets in health and disease and identification of relations between distinct transcriptomic or proteomic profiles and functional properties is of critical significance. Second, systematic study of cell-specific actions *in vivo* will provide us with insights into the cell biological role of these multifunctional cells. Third, considering the co-existence of cardiomyocyte and interstitial pathology in most human myocardial conditions, we need to understand to what extent primary fibroblast activation contributes to dysfunction, arrhythmias and adverse outcome. Because the relative contribution of fibrosis is likely dependent on contextual factors, there is a need to develop strategies for identification of patient subsets with maladaptive or excessive fibrotic responses that may benefit from interventions targeting fibrogenic signalling.

Conflict of interest: none declared.

Funding

N.G.F. laboratory is supported by NIH grants R01 HL76246, R01 HL85440, and R01 HL149407 and by U.S. Department of Defense grants PR151134, PR151029, and PR181464.

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